

RECORDS OF THE MALARIA SURVEY
OF INDIA.



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RECORDS OF THE MALARIA SURVEY OF INDIA.

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THE INDIAN RESEARCH FUND ASSOCIATION

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of hæmatology, protozoölogy and entomology, and the clinical aspects of malaria. It was introduced to avoid the inclusion of more elementary aspects of these subjects into the main course, which were not required by the more experienced students. The preliminary course occupies four days, beginning at 8 a.m. on Wednesday, the 24th April.

The theoretical and laboratory studies of the main course will commence at the King Edward VII College of Medicine at 8 a.m. on Monday, the 29th April, and will be completed on Sunday, the 2nd June. The fee for this stage will be Straits \$ 75.

The practical field studies will follow immediately and for this the candidates will be divided into groups, one of which will study in Malaya, one in French Indo-China, and probably another in Java. They will last approximately 21 days, during which the students will have the opportunity of becoming familiar with the routine of a malariologist and the actual application of anti-larval and other anti-malarial measures to field conditions.

Conditions of admission.—The courses are intended for medical graduates already engaged, or likely to be engaged, in anti-malarial work.

The subscription for the theoretical and laboratory course will be Seventy-five Straits Dollars, and will be received by the Eastern Bureau of the League of Nations at Singapore.

Candidates not attending the preliminary course will be expected to possess a working knowledge of malaria and of the fundamental principles of the contributory subjects, such as hæmatology, protozoölogy and entomology.

Any further information desired will be supplied by the Director of the Eastern Bureau of the League of Nations, 336 River Valley Road, Singapore, to whom applications for admission to the course should be addressed. These should reach Singapore not later than 30th March, 1935, and as only thirty candidates can be admitted, early application is desirable.

The Summary of the Syllabus for the course is appended.

Editor.

SUMMARY OF SYLLABUS.

SECOND INTERNATIONAL COURSE IN MALARIOLOGY.

29th April to 2nd June, 1935.

1. Clinical, 4 hours.

Demonstrations of selected cases and discussion. Relapses and resistance to malaria; provocation of attacks. Induced malaria; delayed manifestations; complications and sequelæ. Determination of what constitutes a cure. Blackwater fever.

2. Pathology, 5 hours.

Pathology of acute malarial fever. The spleen in acute malaria. Pigmentation. Pathology of the metabolic changes in malaria; changes in the blood, urine and special organs. Congenital malaria. Pathology of blackwater fever.

3. Synthetic drugs in the treatment and prophylaxis of malaria, 3 hours.

Natural and synthetic drugs; plasmoquine, Fournneau 710, atebirin, arsenical and other preparations. Pharmacology and toxicology; dosage and administration. Therapeutic effects; effects on the various stages of the parasites; effects on relapses. The present status of synthetic drugs in comparison with quinine and its derivatives.

4. Immunity under special conditions, 6 hours.**5. Protozoology, 14 hours.**

Comparison of staining methods; the enumeration of malaria parasites; cultivation; observation of living parasites; seasonal prevalence of parasites; *Plasmodium ovale*; other indeterminate and doubtful species and varieties in man. Malaria in birds, monkeys and other animals. The action of therapeutic agents on the parasites, especially in chronic cases of malaria.

6. Entomology, 58 hours.

The detailed morphology of anopheline imagines, pupæ, larvæ and ova. The œcology of imagines, larvæ and ova; physical and biotic autœcology, synœcology. The genus *Anopheles*, its classification and affinities; the species and categories below the species; geographical and seasonal distribution; the characteristics of the principal carriers of the world. Susceptibility; influences determining the efficiency of carriers. The determination of species in all stages; the use of keys and original descriptions in a study of the Eastern fauna. The technique of laboratory and field investigations.

7. Epidemiology, 14 hours.

Methods and interpretation of results; measurements of malaria and estimation of its prevalence and intensity; statistical analysis of data. Endemicity, epidemicity and pandemicity, their characteristics and the factors involved. Sources and routes of infection; the infectivity of the patient; the numerical abundance of carriers; topographical, climatic and social factors. Anophelism *sine* malaria. The prediction of epidemics.

8. Control, 36 hours.

(a) Twenty-four hours.—Surveys, urban and rural; field methods for the measurement of malaria. Evaluation of loss from malaria; temporary and permanent measures, their efficiency, cost and financial benefit. Protection from bites; measures against adults; measures against larvæ; biological control. Choice and design of measures in special circumstances; specific control. Organization of anti-malaria work; propaganda.

(b) Six hours.—Surveying and levelling in anti-malaria work. Major and minor engineering works in relation to the cause and prevention of malaria. The construction of sub-soil pipes and the handling of cement in anti-malaria work.

(c) Six hours.—Control in municipal and rural Singapore. The history of the anti-malaria campaign in Singapore.

9. Control—optional, 20 hours.

Demonstrations relating to surveys, methods and results of anti-malaria work, work actually in progress.

10. Special problems in the Netherlands Indies, 5 hours.**11. Special problems in the Philippine Islands, 5 hours.**

12. Special problems and their solution on American Plantations in Malaya, 2 hours
13. Physical properties of oils used as 'larvicides', 2 hours.
14. The work of the Malaria Commission of the League of Nations, 2 hours.
15. Discussion of delegates' own problems, 4 hours.

Total 160 hours out of a possible total of 166 hours

A METHOD FOR CLEANING THE CAPILLARY TUBES USED FOR THE ENUMERATION OF MALARIAL PARASITES IN THE BLOOD.

BY

LIEUT.-COLONEL J. A. SINTON, M.D., D.Sc., I.M.S.
(*Malaria Survey of India, Kasauli*).

[19th December, 1934.]

CHRISTOPHERS, Sinton and Covell (1931) recommended the employment of the capillary tubes used for vaccine lymph, to replace ordinary capillary pipettes, in the method for the enumeration of malarial parasites devised by Sinton (1924). These tubes have been found most convenient and practical for work in the field, but, if hundreds of parasite counts are being done each month, very large numbers of these tubes are used. This caused considerable expense as each tube was used once only. To overcome this disadvantage the following method for cleaning the tubes has been devised, and been found very satisfactory :

(1) The dirty tubes are immersed in a large test-tube containing strong nitric acid.

(2) The test-tube is placed in a water-bath and boiled. This process is continued for about 5 minutes after all signs of blood inside the tubes have completely disappeared*.

(3) The acid is drained off and can be re-used later. A strong current of water is then allowed to run through the test-tube for some minutes to wash out as much acid as possible.

(4) The capillaries are then removed and tied into small bundles with thin rubber bands. Each bundle is slightly thicker than a lead pencil.

(5) About half an inch of the globular end of a rubber teat is cut off, and the ring end is fitted over the nozzle of a water tap. This forms a cone-shaped extension into which the end of a bundle of capillary tubes can be tightly

*The same cleaning action is produced by immersion in the acid for three or four days at room temperature.

fitted. When the water is turned on, it flushes each separate capillary in the bundle. This washing action is allowed to continue for about 10 minutes or so, to ensure complete removal of all traces of acid from the interior of the tubes.

(6) The bundle of capillaries is then removed, shaken to get rid of as much water as possible*, and afterwards immersed in a jar of absolute alcohol for a few minutes, being shaken to remove all bubbles from the interior of the tubes.

(7) The bundle is then taken out of the alcohol, and any excess of fluid shaken off*.

(8) The rubber band is then removed from the bundle, and the capillaries spread out on filter paper to dry. The latter process can be accelerated by placing the tubes in the sun or in a hot chamber.

REFERENCES.

- CHRISTOPHERS, S. R., SINTON, J. A., 'How to do a Malaria Survey'. *Health Bulletin*,
and COVELL, G. (1931) No. 14, *Malaria Bureau*, No. 6, 2nd Ed.
SINTON, J. A. (1924) *Ind. J. Med. Res.*, **12**, pp. 341-346.

*The excess of fluid can be more completely removed, if the bundle be placed in a centrifuge bucket, at the bottom of which is some clean, dry, absorbent cotton-wool, and the handle of the centrifuge revolved a few times.

THE DISPERSION OF ANOPHELINE LARVÆ BY THE FLOW
OF STREAMS, AND THE EFFECT OF LARVICIDES
IN PREVENTING THIS.

BY

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AND

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(*Malaria Survey of India.*)

[4th January, 1935.]

It has long been known that mosquito larvæ may be transported from place to place by the action of currents of water under natural conditions. This belief appears to have been based mainly upon observations on the disappearance of larvæ when breeding places are flooded or flushed out. We have not, however, seen any experimental evidence to show how important a factor this is in the dispersion of larvæ in nature, nor whether such transportation results in a high mortality among these immature insects. It appears generally to be assumed that, when larvæ are washed out by floods, the majority are drowned, or that when they are drifted along by more gentle currents they may fall victims to their natural enemies, especially to fish.

During the course of an experiment upon the effects of Paris-green dusting as a method for controlling the numbers of Anophelines in a village near Karnal, it was noted that, although all water collections within a radius of half a mile of the village were treated regularly every 5-7 days, yet the catch of adult mosquitoes did not diminish in the proportion expected. At first it was suggested that some important breeding place might have been overlooked, or that the work of the distributors of the larvicide was not being performed properly. Careful and repeated inspections showed that these suggestions could be eliminated as a cause of the findings.

Running through the middle of the controlled area is a large shallow channel—the Bhuda Khaira Escape. This takes its origin from the Western Jumna Canal, about 16 miles distant, and is used to receive any excess of

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water from it. It also acts to some extent as a drainage channel at certain seasons. The Escape runs at a lower level than the surrounding country, has high banks, and a sandy bottom. It is about 60-65 feet in width at the point under observation.

At some seasons of the year, there is no flow of water in the channel, and it contains a series of isolated pools along its bed. These pools, which contain much grassy vegetation and algæ (*Spirogyra*), form a vast nursery of Anophelines, chiefly *A. culicifacies* and *A. subpictus*, and occasionally the larvæ of *A. annularis* and *A. barbirostris* are found. During the autumnal months (the main malarial season), the Escape is usually a sluggishly flowing stream about 1 foot deep, with grassy edges and masses of algæ. In spite of the presence of numerous small fish, Anophelines breed in it in enormous numbers, for they are protected from their natural enemies by the vegetation. Villages situated in the vicinity of this channel all show a very high incidence of malaria. When water enters it from the Western Jumna Canal, the stream increases in size and velocity, while during the rainy season (July and August) it may overflow its banks and cause flooding in the vicinity. This flooding is due partly to the water entering from the Canal, and partly to water draining into it from the surrounding country.

In spite of the regular use of Paris green on this channel, along its course inside the controlled area, numerous large Anopheline larvæ and pupæ could be collected in it on the days after each dusting. The very sluggish nature of the flow in this stream, and the abundance of small fish, did not suggest that such large numbers of larvæ could drift into the area from the uncontrolled places outside. As there appeared to be no other obvious explanation, it was decided to investigate the matter by placing a net across the stream and collecting the larvæ caught in it.

TECHNIQUE OF INVESTIGATION.

(a) A strip of muslin about a yard wide, and of a length sufficient to stretch across the stream was obtained. A thin rope was sewn along the top and bottom edges of this strip, leaving free portions at each end. A series of tapes, for tying the strip to the stakes, were fixed along each edge at a distance of 5 feet apart.

(b) A line of bamboo stakes, 5 feet between each, was placed transversely across the stream bed, and the upper edge of the net attached to these, so that this edge was held about 6-8 inches above the water surface. Another series of stakes was placed parallel to the previous line, and about a foot up-stream of them. The lower edge of the net was attached to these at about 8-10 inches below the water surface. The force of the current stretched the net into a series of bag-like bays across the channel. There were 13 of such bays, each 5 foot across, in the portion of the stream being examined. For the purpose of record, these were called Nos. 1 to 13 (*vide* Tables I-XV).

(c) When the net had been placed in position, the material gathered in each bay was collected separately. The net was left in position for 1-4 hours, and collections were made at hourly intervals.

(d) The material floating in each bay was collected separately with a hand net, and washed into a large, white-enamelled, photographic tray

(12 inches by 10 inches), which was marked with the number corresponding to the bay. From each tray the larvæ and pupæ were picked out with a pipette and counted.

(A) THE DISPERSION OF ANOPHELINE LARVÆ BY THE FLOW OF STREAMS.

(i) EXPERIMENTS.

(1) *In the Bhuda Khaira Escape*.—The results of experiments carried out on four consecutive days in this stream are shown in Tables I–IV. The rate of the current at the time was about 300 yards per hour. The temperature of the water varied between 66°F. at 9 a.m. and 78°F. at 12 noon.

From these tables it can be seen that an incredible number of larvæ drifted down this stream into the controlled area. There were a large number of the larvæ in the earlier instars, but a very considerable number of 3rd and 4th stage larvæ, and some pupæ, were present.

The results obtained were most astonishing, more especially as there were numerous small fish in the stream. When the vegetation of the stream was disturbed, so as to drive the larvæ out into the open, these fishes rapidly devoured them. At the time of these experiments, however, there was a large amount of seed, blown from the tall grass along the banks of the stream, floating down upon the surface of the water. This appeared to afford a considerable amount of protection from the fish. The larger catches were usually made in the marginal bays (Nos. 1 and 2, and 12 and 13). This probably depended upon the following factors—(a) the majority of the breeding was along the edges of the stream, and (b) the marginal vegetation afforded better shelter from the fish, while larvæ in the middle of the stream were more exposed to these enemies.

It will be noted that in Experiment No. 3, there was a very sudden increase in the number of larvæ and pupæ captured between 12 noon and 1 p.m. This coincided with a temporary increase in the amount of water and the rate of the current in the Escape, probably due to a sudden influx of water higher up the stream. During this hour, the number of larvæ increased to about 13 times the average found in the same period in Experiments Nos. 1, 2 and 4. As such periodical flushings of the Escape are not uncommon during the malarial season, it shows what an enormous number of larvæ may be swept into the controlled area at such times. The number of larvæ collected is increased because (a) a larger area and a longer length of the stream is flushed in the same period, (b) the rise in the water level washes out detached pools, where the larvæ were previously protected from fish, (c) the greater strength of the current is more liable to make the larvæ drift, and (d) the stronger current is more likely to detach masses of algæ, which float down the stream with their contained larvæ.

Another point of interest is the relatively great increase in the number of pupæ collected in Experiment No. 3. It is well known that a pupa can anchor itself firmly by grasping a piece of vegetation, etc., between its tail and its cephalo-thorax. This power of fixation is probably greater than that possessed

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by the larvæ of any of the species of Anophelines met in these experiments. The results recorded suggest that, with a gentle current, pupæ are less easily dispersed than are larvæ, but with stronger currents both types of immature forms are carried along in the stream.

TABLE I.
(Experiment No. 1.)

(Net placed in position at 9 a.m. on 24th October, 1934.)

Time.	Numbers of larvæ and pupæ collected in the following bays of the net.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	TOTAL.
10 a.m. ..	36	67	16	5	4	5	1	3	11	13	11	23	30	255
11 a.m. ..	45	66	10	4	1	0	63	19	20	12	15	22	40	317
12 noon ..	46	58	12	30	8	14	29	21	27	25	37	14	35	356
1 p.m. ..	133	6	17	60	4	6	0	5	56	11	56	37 (P1)	30 (P1)	421 (P1)
TOTAL ..	290	197	55	99	17	25	93	48	114	61	119	96 (P1)	135 (P1)	1,349 (P2)

Average catch per hour—337 larvæ and 0·5 pupa.

Rate of current about 300 yards per hour. Water temperature at 9 a.m. 66°F. and at 12 noon 78°F.

TABLE II.
(Experiment No. 2.)

(Net placed in position at 9 a.m. on 25th October, 1934.)

Time.	Numbers of larvæ and pupæ collected in the following bays of the net.														TOTAL.
	1	2	3	4	5	6	7	8	9	10	11	12	13		
10 a.m. ..	50	18	13	64	14	21	1	33 (P1)	3	44	5	11	24	301 (P1)	
11 a.m. ..	77	12	30	11	25	1	2	1	2	2	4	5	69	241	
12 noon ..	116	9	36	7	8	23	1	0	0	8	34	41	94	377	
1 p.m. ..	80	13	21	5	1	3	4	1	2	5	9	12	107	263	
TOTAL ..	323	52	100	87	48	48	8	35 (P1)	7	59	52	69	204	1,182 (P1)	

Average catch per hour—296 larvæ and 0·25 pupa.

Rate of current about 300 yards per hour. Water temperature as in Experiment No. 1.

TABLE III.

(Experiment No. 3.)

(Net placed in position at 9 a.m. on 26th October, 1934.)

Time	Numbers of larvæ and pupæ collected in the following bays of the net.													TOTAL.
	1	2	3	4	5	6	7	8	9	10	11	12	13	
10 a.m. ..	25	37	5	2	5	3	13	17	9	23	22	61	39 (P1)	261 (P1)
11 a.m. ..	53	16	12	19	30	25	27	13	27	1	27	10	9	269
12 noon ..	135	93	56	10	4	4	11	88	55	92	60	126	96	830
1 p.m. ..	899 (P5)	2,000 (P10)	303 (P3)	84 (P1)	76 (P1)	22	48	55	57	70	59	112	204 (P1)	3,989 (P21)
TOTAL ..	1,112 (P5)	2,146 (P10)	376 (P3)	115 (P1)	115 (P1)	54	99	173	148	186	168	309	348 (P2)	5,349 (P22)

Average catch per hour—1,337 larvæ and 5.5 pupæ.

Rate of current and water temperature between 9 and 11 a.m. as in Experiment No. 1.

TABLE IV.

(Experiment No. 4.)

(Net placed in position at 9 a.m. on 27th October, 1934.)

Time.	Numbers of larvæ and pupæ collected in the following bays of the net.													TOTAL.
	1	2	3	4	5	6	7	8	9	10	11	12	13	
10 a.m. ..	17	3	0	1	1	15	7	2	5	26	1	30	7	115
11 a.m. ..	18	6	9	5	0	2	3	8	14	1	4	18	5	93
12 noon ..	38	55	37	8	7	9	12	12	13	25	9	22	13	260
1 p.m. ..	120	172	103	91	39	6	2	11	8	5	29	24	45	655
TOTAL ..	193	236	149	105	47	32	24	33	40	57	43	94	70	1,123

Average catch per hour—281 larvæ.

Rate of current and water temperature as in Experiment No. 1.

Several other experiments (Nos. 5, 5c, 6, 6c and 7) were carried out at later dates, as controls for the trials of larvicides to stop the drifting of larvæ. The results of these are shown in Tables V, VIII, IX, XII and XIV.

When these later investigations were made, the quantity of floating grass-seed was negligible. There were, however, very numerous scattered masses of floating algæ (*Spirogyra*), both along the banks and in the middle of the stream. Many of these masses were drifting gently down-stream. On examination, these were found to contain large numbers of Anopheline larvæ, and, when one of them was collected in a bay of the net, the numbers of the larval count were greatly increased. This is the reason why in these experiments, the maximum number of larvæ was not always in the marginal bays. In these later months the number of larvicidal fish present was greatly diminished and was very small.

(2) *In an irrigation channel.*—A similar experiment was carried out in a small irrigation channel near the same village. This channel was about 9 feet wide, had clean-cut edges and a rapid current. No larvæ were collected in experiments lasting 4 hours on each of four consecutive days.

The reason for this was probably the unsuitable conditions for breeding, mentioned above, and also because the channel had been completely dry for some weeks before the experiments were carried out. It is proposed to re-conduct this experiment at another season of the year, when the flow of water is more continuous, as on several previous occasions larvæ have been collected from the edges of this channel.

(ii) DISCUSSION.

There were very considerable variations in the numbers of immature insects collected from day to day and from hour to hour, apart from any great apparent differences in the rate of flow of the stream. The extremes of the hourly catches lay between 179 and 3,989 larvæ (average about 790) and 0 and 56 pupæ (average about 5). In several instances the increase appeared to be due to disturbances of the vegetation in the stream, by cattle coming down to drink and by both men and cattle fording it.

The number of hours during which the net was in position to investigate the larval drift was 24, and during these periods a total of nearly 19,000 Anopheline larvæ and 129 pupæ were collected in it. The majority of these larvæ were *Anopheles culicifacies*, the most dangerous malaria-carrying mosquito of this part of India.

It is interesting to note that this dispersion of larvæ occurred in spite of the presence of numerous larvicidal fish. The immature insects were protected by the floating vegetation and other debris.

(iii) CONCLUSIONS.

(1) The results obtained show that an incredibly large number of living Anopheline larvæ may be carried along by the current of streams, if conditions be favourable.

(2) Even in the presence of numerous larvicidal fish, aquatic vegetation and floating debris may protect large numbers of such larvæ from destruction.

(3) This drifting of larvæ may have a very serious effect in disturbing the results of anti-mosquito measures. 1

(B) THE EFFECTS OF LARVICIDES UPON THE DISPERSION OF LARVÆ.

(a) THE EFFECTS OF OILING.

(i) EXPERIMENTS.

The number of larvæ drifting down the Bhuda Khaira Escape was again determined, and found to average 2,012 per hour (*vide* Table V).

As noted above, at this time the amount of floating grass-seed was negligible, but there were numerous masses of algæ being drifted along in the current. These contained large numbers of larvæ. As such masses when drifting near the edges of the stream tended to become stranded; the moving algal masses were more numerous towards the middle of the stream.

TABLE V.

(Experiment No. 5.)

(Net placed in position at 9-30 a.m. on 17th November, 1934.)

Time.	Numbers of larvæ and pupæ collected in the following bays of the net.													TOTAL.
	1	2	3	4	5	6	7	8	9	10	11	12	13	
10-30 a.m.	66 (P1)	59	163	90	65	77 (P3)	31	60	25	51 (P1)	37 (P1)	86	189 (P1)	999 (P7)
11-30 a.m.	147 (P2)	123 (P1)	200 (P3)	54	600 (P4)	400	300	149 (P1)	28	36	200	300 (P4)	400	2,937 (P15)
12-30 p.m.	160 (P1)	350 (P2)	190 (P1)	27	297	400	290	39	17	9	25	97	199 (P1)	2,100 (P5)
TOTAL	373 (P4)	532 (P3)	553 (P4)	171	962 (P4)	877 (P3)	621	248 (P1)	70	96 (P1)	262 (P1)	483 (P4)	788 (P2)	6,036 (P27)

Average catch per hour—2,012 larvæ and 9 pupæ.

Rate of current about 250 yards per hour. Water temperature at 9-30 a.m. 58°F.

Immediately after the test catch was completed, the net was removed. Two large 'oil-balls'—sacks filled with sand impregnated with waste lubricating oil*—were then placed in the water, one at each margin of the stream, about 100 yards up-stream of the previous position of the net. These were left to act over-night.

About 3 gallons of oil was used in each ball.

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Next morning, 20½ hours later, the net was again stretched in position. A thin film of oil was apparent upon the surface of the water. The results of catches during three hours are given in Table VI.

TABLE VI.

(Experiment No. 5a.)

(Net placed in position at 9-30 a.m. on 18th November, 1934, 20½ hours after oiling.)

Time.	Numbers of larvæ and pupæ collected in the following bays of the net.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	TOTAL.
10-30 a.m.	20 (P2)	12	4	9	1	8	10	12 (P1)	20 (P2)	27	15	30 (P1)	8	176 (P6)
11-30 a.m.	15 (P1)	5 (P1)	2	1	2	4	10	11	2	20	1 (P2)	18 (P3)	25	116 (P7)
12-30 p.m.	3	5	18	12	0	8 (P1)	2	5 (P1)	3	2	4	7 (P1)	2	71 (P3)
TOTAL ..	38 (P3)	22 (P1)	24	22	3	20 (P1)	22	28 (P2)	25 (P2)	49	20 (P2)	55 (P5)	35	363 (P16)

Average catch per hour—121 larvæ and 53 pupæ.

Rate of current as in Experiment No. 5. Water temperature at 9-30 a.m. 54°F. and at 1 p.m. 70°F.

These results show a very marked diminution in the numbers of larvæ, and may be summarised as follows :—

	Larvæ.	Pupæ.
(1) Average hourly catch before oiling ..	2,012	8.6
(2) Average hourly catch 20½ hours after oiling ..	121	5.3
(3) Percentage decrease ..	94.8	35.0

When the site of operations was re-visited 10 days later, it was found that one of the oil-balls had been stolen, but as the oil-impregnated sand had been dumped into the river bed, there was still a slight film of oil floating down the stream near one bank.

The net was again placed in position, and collections made during a period of 4 hours (*vide* Table VII). On account of the variations in larval drift noted above, the net was also stretched a few yards up-stream of the oil-balls, and left in position for 2 hours (*vide* Table VIII).

TABLE VII.

(Experiment No. 5b.)

(Net placed in position at 9 a.m. on 27th November, 1934.)
(Ten days after oil-balls placed in stream.)

Time.	Numbers of larvæ and pupæ collected in the following bays of the net.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	TOTAL.
10 a.m. ..	0	0	0	0	2	0	0	0	0	(P1)	1	0	1	4 (P1)
11 a.m. ..	2	(P1)	5 (P1)*	3	3	7	4	3	2	0	8	0	4	41 (P2)
12 noon ..	3	0	2	0	0	0	1	1	2	3	2	4	4	22
1 p.m. ..	1	3	6	0	0	1	0	1	3	0	1	0	2	12
TOTAL ..	6	3 (P1)	7 (P1)	3	5	8	5	5	7	3 (P1)	12	4	11	79 (P3)

Average catch per hour—20 larvæ and 0·8 pupa.

TABLE VIII.

(Experiment No. 5c.)

(Net placed in position at 10 a.m. on 28th November, 1934, in an un-oiled portion of the stream.)

Time.	Numbers of larvæ and pupæ collected in the following bays of the net.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	TOTAL.
11 a.m. ..	44 (P3)	49	40 (P1)	5	0	2	3	2	6 (P1)	44	7 (P1)	37	10	219 (P6)
12 noon ..	170 (P2)	200 (P1)	51 (P1)	5	8	3	15	11	5	6	3	10	7	494 (P4)
TOTAL ..	214 (P5)	249 (P1)	91 (P2)	10	8	5	18	13	11 (P1)	50	10 (P1)	47	17	743 (P10)

Average catch per hour—371 larvæ and 5 pupæ.

The comparative results of Experiments Nos. 5b and 5c, to determine the numbers of larvæ entering and leaving an oiled area, may be summarised as follows :—

	Larvæ.	Pupæ.
1. Average hourly catch above oiled area ..	371	5
2. Average hourly catch (10 days) after oiling ..	20	0·8
3. Percentage decrease	94·6	84·0

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The results of this experiment show that a very appreciable reduction in the number of larvæ drifting into a controlled area can be obtained by the use of continuous oiling in the form of oil-balls.

It is well known that films of oil penetrate floating masses of algæ with difficulty. So it seemed possible that larvæ embedded in such masses might often escape destruction by the oil. A further experiment was carried out to determine whether the method of oiling described above could be improved upon. It was thought that, if a floating boom of some material were placed across the stream, it would be possible to stop a large number of the algal masses floating into the controlled area, and at the same time the accumulation of the oil-film behind the boom might have a more destructive action.

In deciding upon the nature of the boom, it was essential, as we were dealing with an undisciplined population, that the materials chosen should be such that there would be little or no inducement for the local people to steal them. Accordingly the boom was constructed from materials obtainable on the spot. The dried stems of the ten-foot-high grass growing on the banks of the stream were bound together with fibrous portions of their leaves. In this way a long cable about $2\frac{1}{2}$ inches in diameter and 65 feet long was made.

While this was being prepared, the net was stretched across the stream about 50 feet below the proposed position of the boom. The results of the collection during one hour are shown in Table IX.

TABLE IX.
(Experiment No. 6.)

(Net placed in position at 10 a.m. on 24th December, 1934.)

Time.	Numbers of larvæ and pupæ collected in the following bays of the net.												
	1	2	3	4	5	6	7	8	9	10	11	12	TOTAL.
11 a.m. ..	21 (P1)	14	14	4	8 (P1)	11	15	9	18	35 (P1)	13 (P1)	17	179 (P4)

Catch per hour—179 larvæ and 4 pupæ.

Rate of current about 180 yards per hour. Water temperature at 10-30 a.m. 64°F.

Of the larvæ collected in Experiment No. 6, it was found that 7 were dead. This, and the comparatively small number of larvæ found, was probably due to the continued action of the Paris green applied two days previously (*vide* Table XV, Experiment No. 7a).

The boom was placed in position by attaching it to a stake on either bank, while several smaller stakes were placed behind it across the bed of the stream, to support it against the force of the current. It was not attached to these small stakes, so that it could rise and fall with the water in the stream. When in position it lay as two curves with the concavities up-stream.

The boom was placed in position at 11-45 a.m. and the net, which had been raised after Experiment No. 6, was again lowered. This was done to determine whether the boom by itself had any temporary effect in checking the drift of the larvæ. The results are shown in Table X.

TABLE X.

(Experiment No. 6a.)

(Net placed in position at 11-45 a.m. on 24th December, 1934, fifty feet below the boom.)

Time.	Numbers of larvæ and pupæ collected in the following bays of the net.												TOTAL.
	1	2	3	4	5	6	7	8	9	10	11	12	
12-45 p.m.	5	2	8	3 (P1)	1	3	7	12	6	10	3	3	63 (P1)

Catch in one hour—63 larvæ and 1 pupa.

As in the previous experiment, 8 of the larvæ collected were dead. If the results of Experiment No. 6a be compared with those of No. 6, it is seen that the boom by itself has some action in stopping the drift of the larvæ. During the period of observation several masses of floating algæ were seen to be held up by it. To confirm the obstructive action of the boom, the floating material behind it was collected one hour after it had been placed in position. This material was found to contain 173 larvæ (19 dead) and 2 pupæ. If these figures be added to those of Experiment No. 6a, they show that about 75 per cent of the larvæ drifting down-stream were stopped, temporarily at least, by the boom.

Two oil-balls, each about the size of a foot-ball, were then made from palm fibre and other material found on the bank of the stream. Each of these was impregnated with about half a gallon of waste lubricating oil, and placed in the water at each edge of the stream, about 100 feet above the boom. The water surface between the oil-balls and the boom was sprinkled with another gallon of oil. When this was finished a large amount of the oil had collected in the curves of the boom.

When the spot was re-visited next day, both oil-balls were working and there was a thick layer of black oil behind the right portion of the boom, but not on the left. It was found that small wavelets on the surface of the water caused the oil layer to slop over the top of the boom and drift down-stream. This was especially noticeable when cattle were watered in the vicinity of the boom. This action probably accounted for the absence of a thick oil layer in the left portion of the boom. About seventy hours after the boom had been placed in position and oiling carried out, the net was again stretched about 50 feet below the boom. The results of collection after 1 hour are shown in Table XI.

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TABLE XI.
(Experiment No. 6b.)

(Net placed in position at 10 a.m. on 27th December, 1934, i.e., 3 days after the oiling.)

Time.	Numbers of larvæ and pupæ collected in the following bays of the net.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	TOTAL.
10 a.m. ..	1	0	3	0	3	3	5	4	4	1	2	2	44	72

Catch in one hour—72 larvæ.

As a control for this experiment, the net was again placed in position about 20 feet up-stream of the oil-balls, to see whether the diminution in larval numbers might not be a natural phenomenon. The result of this collection is shown in Table XII.

TABLE XII.
(Experiment No. 6c.)

(Net placed in position at 11-20 a.m. on 27th December, 1934, about 20 feet above the position of the oil-balls.)

Time.	Numbers of larvæ and pupæ collected in the following bays of the net.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	TOTAL.
12-20 p.m.	6	6	48 (P3)	98 (P3)	101 (P6)	53 (P5)	70 (P2)	192 (P3)	387 (P9)	129 (P4)	367 (P7)	173 (P2)	457 (P14)	2,087* (P58)

Catch in one hour—2,087 larvæ and 58 pupæ.

* It will be observed in this experiment and the previous one that the maximum larval catch occurred towards the right of the table, i.e., the left bank of the stream. This is probably accounted for by the greater velocity of the current at this side. In the case of Experiment No. 6c, the small numbers near the right bank were possibly influenced by some oil still soaking out of the sand used in the earlier oiling experiment.

The results of this experiment on oiling may be summarised as follows :—

	Larvæ.	Pupæ.
(1) Hourly catch above oiled area 2,087	58
(2) Hourly catch below boom and oiled area 72	0
(3) Percentage decrease after 3 days 96.6	100

After the boom and the oil-balls had been in position for 5 days the situation was again examined. There was still some thick oil behind the right curve of the boom, but not on the left. The oil-balls were only acting very feebly, and some living larvæ could be found between the balls and the boom.

A large amount of algæ and other floating debris had accumulated behind the latter, and long pieces of the algæ were trailing down-stream under it.

The net was fixed near the same position as in Experiment No. 6 (*i.e.*, about 50 feet below the boom), and the results of the catch are given in Table XIII.

TABLE XIII.

(Experiment No. 6d.)

(Net placed in position at 9-50 a.m. on 29th December, 1934, 5 days after

Time.	Numbers of larvæ and pupæ collected in the following bays of the net.												TOTAL.
	1	2	3	4	5	6	7	8	9	10	11	12	
10-50 a.m.	65	62 (P1)	24	16	19 (P1)	14	14	23	29	24 (P1)	13	72	375 (P3)

Catch in one hour—375 larvæ and 3 pupæ.

Rate of current about 200 yards per hour. Water temperature 54°F.

(ii) DISCUSSION.

These experiments show that a floating boom will cause a diminution in the numbers of larvæ drifting down-stream. This action appears to be due mainly to its effect in stopping masses of floating vegetation and debris, among which the larvæ collect and are protected against their natural enemies. If, however, large amounts of such material be floating down-stream, a boom of the diameter used in our experiments (2½ inches) appears to allow a certain quantity of such material to pass under it, when the pressure behind becomes great. A boom of a larger diameter would probably be more effective. It also appears advisable that, every 5 days or so, the debris accumulated behind the boom should be sprayed with oil and then removed. This oiling would kill any larvæ which had escaped the effects of the oil-balls, and which might be washed down-stream when the debris was being removed. The removal of the collected material would relieve the pressure behind the boom, so making it less likely for debris, etc., to pass under it, and would also make conditions more favourable for the oil which accumulates behind it.

It was also noted that with the thin boom used here, disturbances in the water were likely to cause the oil collected behind it to slop over and drift down-stream. This might be prevented to a greater extent if a thicker boom, standing higher out of the water, were used.

In localities where bamboos grow plentifully, such a thicker boom would probably be easily, quickly and cheaply made on the spot.

(iii) CONCLUSIONS.

The drifting of larvæ into a controlled area can be greatly diminished by a continuous application of oil, as by the use of oil-balls.

A floating boom placed across the stream reduced the amount of debris and the number of larvæ drifting into controlled area, and made the action of oiling more prolonged and effective, under the conditions of our experiments.

(b) THE EFFECTS OF DUSTING WITH PARIS GREEN.

(i) EXPERIMENTS.

After the numbers of larvæ drifting into the oiled area were determined in Experiment No. 5c (Table VIII), the stream above the net was dusted with a 5 per cent mixture of Paris green, for about 100 yards up-stream. The amount of larvicide used was in the proportion of $\frac{1}{2}$ lb. to the acre. The net was left in position and a larval collection made 3 hours later. At this time a large amount of the floating larvicide had accumulated in the bays of the net.

The total catch after 3 hours was only 5 living larvæ and 3 pupæ. There were, however, large numbers of dead larvæ, and it was uncertain whether these had drifted into the net in a moribund state, or had been killed *in situ* by the film of larvicide collected immediately behind the net.

On a later date, the number of larvæ drifting down-stream was again determined (Experiment No. 7, Table XIV), and the net removed. The stream was then dusted, as in the previous experiment, and the net placed in position again after 3 hours. The results of this later collection are shown in Table XV.

TABLE XIV.

(Experiment No. 7.)

(Net placed in position at 10 a.m. on 22nd December, 1934.)

Time.	Numbers of larvæ and pupæ collected in the following bays of the net.												TOTAL.
	1	2	3	4	5	6	7	8	9	10	11	12	
11 a.m. . .	62	31	101 (P1)	22 (P2)	35	34 (P1)	59	35 (P3)	60	218	99	30	786 (P7)

Catch in one hour—786 larvæ and 7 pupæ.

Rate of current about 220 yards per hour. Water temperature at 11 a.m. 61°F.

TABLE XV.

(Experiment No. 7a.)

(Net placed in position at 2-15 p.m. on 22nd December, 1934, about 3 hours after Paris green was applied.)

Time.	Numbers of larvæ and pupæ collected in the following bays of the net.												TOTAL.
	1	2	3	4	5	6	7	8	9	10	11	12	
3-15 p.m. . .	3 10	2 7	5 8	4 2	4 2	4 2	2 6	4 2	3 7	1 1	1 2	0 0	33 living. 49 dead.

Catch in one hour—33 living larvæ and 49 dead ones, a total of 82.

A comparison of these two tables shows that at the end of 3 hours the Paris green had a very marked action in reducing the number of larvæ floating down the stream. When a collection was made two days later, it was found that the numbers of larvæ drifting down-stream had again increased (*vide* Table IX), while a collection made 5 days after the larvicide had been applied gave almost the largest hourly catch which has been recorded in our experiments (*vide* Table XII).

(ii) DISCUSSION.

It was found that dusting with Paris green had a marked effect in reducing the number of drifting larvæ collected three hours afterwards, but 5 days later, which might be taken to be the normal interval between routine dustings, the larvæ collected were as numerous as previously.

This was to be expected, because, while Paris green will have a marked immediate effect on the larvæ in the vicinity, it would soon drift down-stream and would sink in a few days. For these reasons, fresh larvæ entering the control area would be unaffected, indeed the action of Paris green in penetrating drifting masses of algæ would be less than that of oil under these conditions.

It is possible that the effects of Paris green might be prolonged for a day or so by the introduction of a boom to prevent the larvicide from drifting down-stream, but this would not prevent it from sinking.

(iii) CONCLUSIONS.

Paris green has a rapid killing action upon larvæ drifting in the area dusted. Its effects are, however, of comparatively temporary duration, and, if used in a routine fashion every 5 days or so, this method of destruction of drifting larvæ would appear to be relatively ineffectual, under the conditions of our Experiment.

THE VERTEX AND THE PHALLOSOME LEAFLETS OF *ANOPHELES MACULIPENNIS* MEIGEN.

BY

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[10th January, 1935.]

WE now know for certain that *A. maculipennis* is not a homogeneous species, but a collection of different varieties. Intensive research is being carried on with a view of finding out morphological differences which would enable us to differentiate these races or varieties without resorting to the tedious process of breeding them from their eggs. The possibility that the six recognised varieties of this species might be separable on points other than the hypopygial morphology, led me to examine carefully—

- (a) the chaetotaxy of the vertex, and
- (b) the morphology of the leaflets of the phallosome.

Differences in the chaetotaxy of the vertex of some species of *Anopheles* have been noted by Christophers (1933). The leaflets of the phallosome have also been used by King (1931) and by Christophers (1933) in working out varietal distinctions. It seemed probable, therefore, that there might be differences in the above two characters in the varieties of *A. maculipennis*. Even if this were not found to be the case, there was the fact that neither of these characters appeared to have been described in this species.

I. CHÆTOTAXY OF THE VERTEX.

TECHNIQUE.

The study of the vertex was first directed to the inter-ocular portion. The head of the female was first detached, and, after being allowed to soak for 15 minutes in 90 per cent alcohol, it was passed into a solution of caustic potash in which it was left overnight. Next morning it was passed through two changes of water, and then left in a very weak carbol-fuchsin solution.

On the third day it was passed through various strengths of alcohol (30, 50, 70 and 90 per cent) into absolute alcohol, and finally into amyl alcohol. The dehydrated specimen was then kept in xylol until it was dissected.

Dissection was carried out in Canada balsam on a slide. A cut was made through the eyes and the base of the clypeus, and the inter-ocular vertex isolated. After arranging the specimen, it was left in a covered space (to avoid dust) for 2-3 hours. Finally the specimen was prepared by scraping away the outer edges of hardened balsam, a fresh drop of balsam added and a cover-slip applied.

An examination of specimens prepared in this manner gave a very erroneous impression of the differences present. Whereas in *A. maculipennis atroparvus* only 6 chætal scars could be discerned, in *A. maculipennis messeae* there were at least 12 to 15. It was, therefore, decided to prepare specimens in which all the scales, etc., were preserved in position. This was rather a difficult procedure, because manipulation through the various alcohols entailed more handling, and consequently a greater chance of injury, while Canada balsam made the specimens too brittle to leave all the scales intact. Preparations in chloral gum afforded a solution of the problem. Better preparations were, however, obtained in carbolic acid, but, unfortunately, the non-permanency of these specimens was a great drawback.

CHÆTOTAXY.

The characters found in the last two sets of preparations are shown in Plate I, fig. 1. The structures seen are—

(1) *The vertical chætæ*—These are of two kinds—large and small. The large vertical chætæ (Plate I, figs. 1 and 2, 1L—7L) usually number 7 or more. They lie between the eyes on either side of the middle line and end in a chæta just posterior to the angular bend of the eye margin. This series of large vertical chætæ is continued backwards as three, four or more small chætæ (Plate I, figs. 1 and 2, 1S—6S) at intervals behind the eye margin until they reach the larger ocular chætæ (Plate I, figs. 1 and 2, 1O). The latter usually number 6 or 7, only the first of which is shown in the figures.

(2) *Ocular scales* (Plate I, fig. 1).—These are small narrow, fusiform or lanceolate scales arising from small bases. They form one series along the eye margin, and another series with a V-shaped arrangement towards the middle line of the head.

(3) *Forked scales* (Plate I, fig. 1).—The so-called 'forked scales' cover the occiput, and extend up to the posterior large vertical chæta.

Examination of specimens of *A. maculipennis atroparvus*, *messeae* and *typicus* showed that this was the general arrangement in all, except that the small chætæ are comparatively scantier in *atroparvus* than in *messeae* and *typicus*.

II. THE LEAFLETS OF THE PHALLOSOME.

TECHNIQUE.

The specimens were prepared in chloral gum by the method of King (1932). The phallosome was split and the leaflets flattened out to show their true shape and characters.

PLATE I.

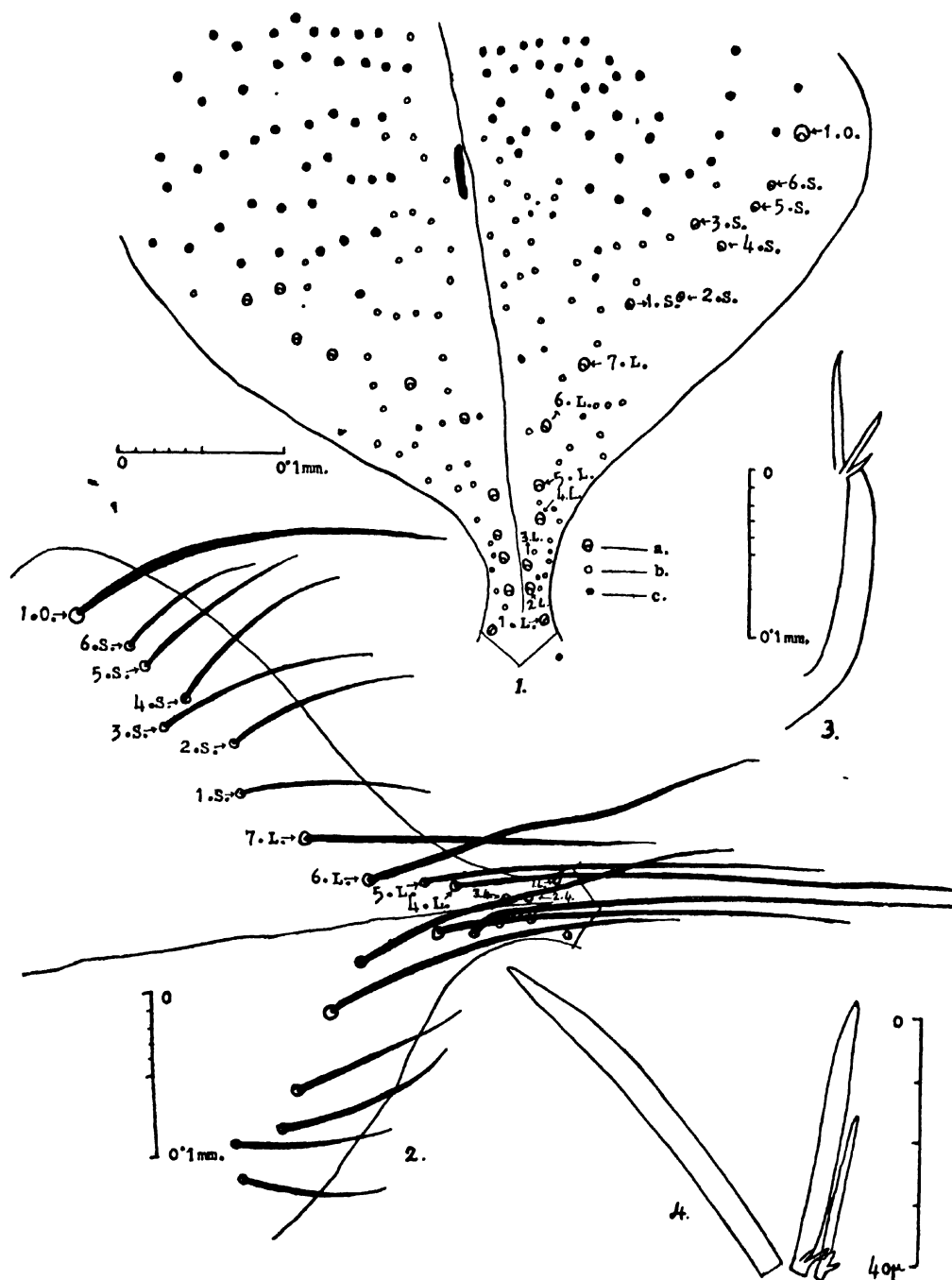


Fig. 1.—Vertex of *A. maculipennis messeae* from Albania, with hairs and scales removed to show distribution of scars—(a) scars of vertical chaetae; (b) scars of ocular scales; (c) scars of forked scales.

Fig. 2.—As in fig. 1 but showing vertical chaetae.

Fig. 3.—Phallosome of *A. maculipennis atroparvus* from London.

Fig. 4.—Drawing on larger scale of leaflets in fig. 3.

MORPHOLOGY.

The phallosomes of *A. maculipennis atroparvus*, *A. m. messeae* and *A. m. typicus* were found to be of the form of somewhat flattened rods (Plate I, fig. 3). They number three on each side, and sometimes have a minute additional basal one. They decrease very regularly in length from the larger inner leaflet by in about the proportion of 5/8th and 5/16th of this respectively. They are smooth and devoid of serrations. At the extreme base of the second and third leaflets is a fine spicule (Plate I, fig. 4). No significant difference could be found in the varieties studied.

CONCLUSIONS.

This report is a preliminary one, so one cannot arrive at any definite conclusions. *A. maculipennis* seems to conform to 'Rassenkries', where diversity of physiological characters is greater than diversity of forms.

ACKNOWLEDGMENTS.

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THE EFFECT OF HEAT AND ATMOSPHERIC HUMIDITY ON ALL STAGES OF *CULEX FATIGANS*.

BY

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[14th December, 1934.]

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I. INTRODUCTION.

SINCE the rôle of insects in the domain of disease was discovered by pioneers like Patrick Manson and Ronald Ross, research has been directed mainly towards routine blood examination; the capture, identification and dissection of insects involved; the mapping out of areas of breeding; and the annihilation of insects concerned in disease, at all stages of their development. These investigations have extended our knowledge of parasitology so far that there appears to be little room for further advance in these directions. We are, however, still ignorant of many of the factors responsible for the occurrence of insect-borne diseases, especially when they appear in epidemic form. The author feels that the cause of these epidemics would be better understood if we knew more of the effect of climate upon biting insects. But by climate we must understand the actual microclimate in which the insects live. Work in the field, on insects and climate, is synthetic. It must be accompanied, if not preceded, by work in the laboratory, which is analytic. By this means not

only do the field observations become better understood but also better correlated. Here one is inclined to endorse the views of Buxton (1933) who rightly remarks that 'much of the work on the relations of insects to temperature is fragmentary. For one species we know the temperature which is fatal to the egg on a five-minute exposure; for another the temperature at which the adult will not suck blood or lay eggs. It appears that no thorough investigation has been made of the effect of temperature on any insect of medical importance'.

It is the purpose of this paper to consider the evidence made available in the laboratory regarding the effect of temperature on various stages of development of *Culex fatigans*, and the influence of atmospheric humidity on the thermal death point of the adult mosquito. Unless such a critical study has been made in the laboratory, certain prevalent ideas which appeal to common sense go unrefuted. For example Mayne (1930), quoting Barraud, remarks that 'mosquito lives at the temperature of wet bulb thermometer in the hygrometer. It is a moist animal protected by a thick cuticle traversed by innumerable trachea which are in direct communication with every cell within, and outside air by spiracles. Therefore owing to evaporation through these tubes temperature of mosquito must be at wet bulb thermometer in an analogous situation'. This is an impossible statement, because the largest female adult weighing 3 mg. has a surface area of about 14.2 sq. mm. If it kept its body 5°C. cooler than air for one hour it would absorb nearly 0.8 calories, to get rid of which about 1.6 mg. of water would need to be evaporated. And since this is over 50 per cent of the insect's weight, it is impossible for it to survive so great a loss (cf. Mellanby, 1932). One may indeed claim that, if our sanitary measures are to be directed against a particular species of insects, we ought to possess considerable knowledge of the effects of climate upon them. *Culex fatigans* is of special importance to us in India as it is the carrier of *Filaria bancrofti*, the cause of filariasis. No apology is therefore needed for work which may at first glance appear to be unrelated to medicine.

II. METHODS OF BREEDING.

The adults of *Culex fatigans* which were used in these experiments were bred from a stock brought from India by Col. Sir S. R. Christophers, and which had been kept for about two years in this laboratory. Feeding of larvæ, collection of pupæ, care of feeding females on the canary, separation of gorged females in separate cages, attention to laying of egg rafts were all necessary in order to have a large number of insects available for the experiments.

Larvæ were kept in enamelled metal basins, 13 inches in diameter and 6 inches deep, in hay infusion through which compressed air was forced by means of a series of T-shaped glass pieces and rubber connections. Hay infusion was prepared by pouring boiling water over hay. It was used when 48 hours old and was renewed every day. Pupæ were separated by pouring mixed larvæ and pupæ into a tall glass cylinder and inserting a circular strainer at the top. This separated the majority of pupæ and those which remained were picked out with a pipette. These pupæ were placed in enamelled iron bowls inside a mosquito cage for emergence. Very few adults failed to emerge. Gorged mosquitoes were transferred to a separate cage, as this allowed of their being kept at a regulated temperature and of a cleaner collection of egg rafts.

III. MANIPULATION.

Insects were collected into a celluloid tube (*vide* Fig. 1), $4\frac{1}{4}$ inches long and 1 inch in diameter, with tapering inverted margins ending in a narrow inlet,

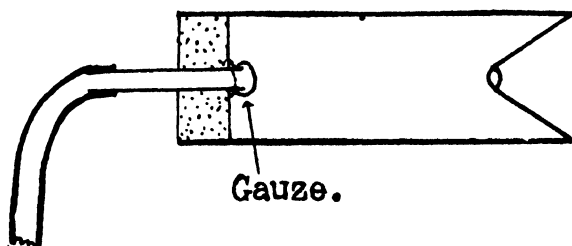


Fig. 1.

but a wide collecting area at one end and a wide mouth at the other end. The latter was corked, and through it a narrow glass tube was passed. This tube was connected with the mouth piece by a piece of rubber tubing. The insects were prevented from passing right through by mosquito netting, which was tied round the end of the glass tubing inside the celluloid chamber.

For transference of adults, the device of Lewis (1933) (*vide* Fig. 2) was used with slight modification*.

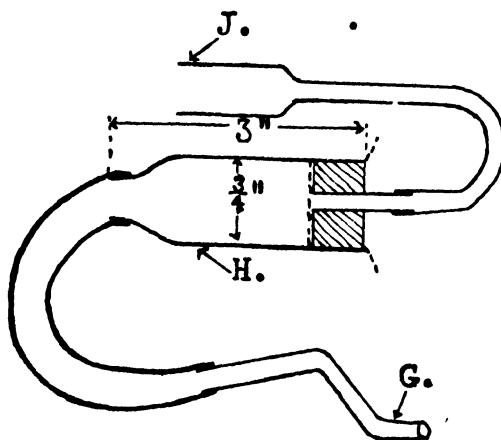


Fig. 2.

Experiments on larvæ and pupæ were carried out in a tank of water, heated electrically and controlled thermostatically to within 0.2°C . The contents of the tank were stirred with compressed air. Larvæ were transferred by means of a pipette to glass beakers filled within 1 inch of the brim with diluted hay infusion. This prevented starvation during the experiment. A thermometer was placed in each beaker to record accurately the temperature to which larvæ and pupæ were being exposed.

* *Vide* description given by Lewis (1933).

Since the adults live in air one had to work at 4 different controlled humidities. The tank was the same as mentioned above. The apparatus used for exposing adults to high temperatures at various humidities was similar in essentials to that previously used by Heattie (1928) and by Buxton (1931), and modified by Mellanby (1932). It is shown in Fig. 3.

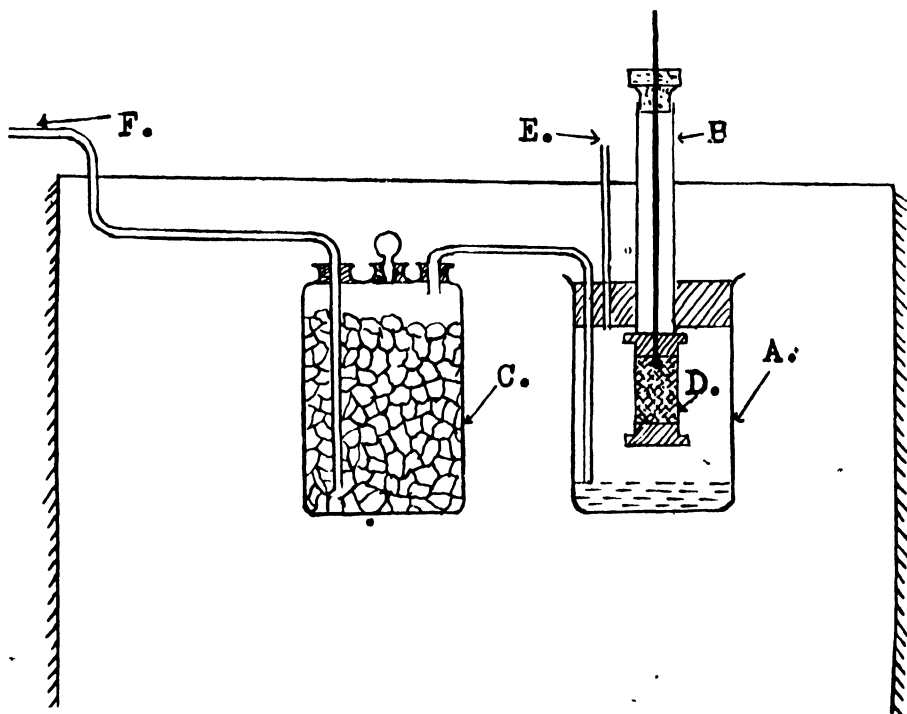


Fig. 3*.

The insects were placed in a bottle (A), which was kept well submerged below the water. It was necessary to do so, because, even if only the neck of the bottle were above the surface, the air in 'A' might be several degrees cooler than the water. The total submergence of 'A' made it more difficult to introduce the adults. A broad glass tube (B) was introduced into the middle of 'A'. A small phosphor-bronze cage (D), containing the insects, was put down the tube 'B' on a thin glass rod. The humidity inside 'A' was controlled by means of a layer of an appropriate mixture of sulphuric acid and water at the bottom (*vide* appendix), and fresh air could be blown through 'C', another Woulff's bottle containing pumice and the same acid as 'A'. At the outlet 'E', there was a piece of capillary glass tube to prevent undue backward diffusion. This method of introducing mosquitoes in containers has certain advantages. There is no need to anæsthetise the insects, and they are in intimate contact with the air.

*The author in this plate has drawn the container 'D' too large. This should be of such a diameter as to pass easily through the tube 'B'.—*Editor.*

At first it looks difficult to see how mosquitoes could be got into wire gauze containers without anæsthetising, but Fig. 2 shows how this is easily done. The twice bent tube 'G' is introduced into the mosquito cage, and the required number of male and female insects are drawn into the glass container 'H' by sucking at 'J'. A fast current of air at 'G' facilitates this manœuvre. The bent tube 'G' is then inserted into the wire gauze container 'D' which is taken out of bottle 'A' through glass tube 'B' for this purpose (Fig. 3), and the mosquitoes blown into it. I found it easier to cover the container with a small piece of card-board, through the centre of which a hole was bored to admit the terminal part of the bent tube 'G'. If mosquitoes are blown with a fair amount of force, they are made sufficiently inactive to permit replacing the card-board by the cork without allowing any to escape. The amount of force required is soon learnt by experience. If too much force be used the insects become permanently damaged, while if insufficient force be used they remain active and fly away. After the insects are introduced into the container 'D', it is corked and passed down the bulb 'B' into bottle 'A'. Air was gently blown at 'F' at the beginning of each experiment. Four pairs of Woulff's bottles (each pair with different humidity of 0, 30, 60 and 90 per cent) were inserted parallel in a 'Meccano' frame. This ensured parallel experiments at different humidities, but at one temperature. The results were thus strictly comparable. Six mosquitoes (three males and three females) were experimented with in each bottle at a time. The main apparatus was checked from time to time. The degree of humidity inside the bottles was checked by aspirating small samples of air with an India-rubber tube and estimating their humidity by means of dewpoint. The dewpoint apparatus was kindly lent to me by Professor Buxton, and is the same as mentioned by him in his work (Buxton, 1933).

IV. EXPERIMENTS.

(i) EFFECT OF RAISED TEMPERATURE ON EGGS.

Batches of at least 20 eggs were taken at a time. The egg rafts employed were 12 to 24 hours old.

Referring to Chart I it will be observed that the limiting lethal range is very sharply defined. At and below 39.8°C. all eggs hatched, while above this temperature, none hatched. Each of these experiments was done with a control, which was obtained from the same egg raft. Experiments in which the control did not hatch were discarded. The extreme uniformity of the results is remarkable, and was probably due to the fact that the egg rafts were uniform in age. Eggs appear to resist a higher temperature than the larvæ. At first we supposed that the egg floating on the surface was exposed to a lower temperature than that recorded by a thermometer with its bulb immersed in the beaker. To test this suggestion similar experiments were conducted in small 1 inch diameter tubes, which were half filled with water and eggs put in them. These were inverted in beakers. The air in the upper parts of these inverted tubes, to which eggs were all the time exposed, was thus kept at the temperature of water. No difference was found in several sets of these experiments, the lethal limit of temperature constantly remaining at 39.8°C. It was noticed that the eggs which failed to hatch after exposure to lethal temperature did not uncap. Whether this was due to certain changes inside the egg shell, or in the egg shell itself, is problematical.

(ii) EFFECT OF RAISED TEMPERATURE ON LARVÆ.

There are so far only two papers dealing with the effect of raised temperature upon larvæ of mosquitoes (Macfie, 1920; Wright, 1928). In both of these the usual time of exposure was 5 minutes. Macfie's experiments were conducted with *Aedes argenteus*, those of Wright with *Anopheles bifurcatus*, *Theobaldia annulata*, *Aedes detritus*, and *Culex pipiens*. I have attempted to increase our knowledge of the subject by exposing insects fully for one hour, and occasionally for longer periods.

In the following experiments, batches of 10 larvæ were exposed at a time, and the same experiment was repeated 2 to 3 times. The larvæ used were in the 4th instar. It will be seen from Chart I that below 36°C. larvæ do not seem to be affected by heat; between this temperature and 38°C. a varying number did not survive an exposure of 1 hour; temperatures of 38°C. and above for one hour killed all the larvæ. Below 37°C. over 50 per cent survived, while above 37°C., over 50 per cent died. In the vicinity of 36°C. the temperature started showing some effect* after one hour exposure; in the vicinity of 37°C. the larvæ appeared inert†; and in the vicinity of 38°C. they were apparently dead‡. Some of those larvæ, which looked apparently dead after exposure, revived after a lapse of 1 to 2 hours. Only those larvæ, which survived 24 hours and appeared normal, were considered as having survived. It was observed that, in the vicinity of the lethal temperature, when larvæ were first introduced they sank right to the bottom as if they had become dazed, and lay quiescent for about 15–20 seconds before they wriggled up to the surface. On Dr. Mellanby's suggestion that asphyxiation may have been the cause of death, the experiments were repeated, the larvæ being kept within 1 inch of the top by gauze. This made no difference, and I concluded that death was due to heat and not asphyxia. One more fact observed was that most of the larvæ which died had turned fawn creamy colour, in contrast with pupæ which under similar conditions became brown. An explanation of this phenomenon was offered by Dr. Wigglesworth. According to him the brown colour which appeared in the dead pupæ was perhaps due to an increase in tyrosin in the pupal stages—an increase associated with the development of pigment in the adult, which is not present during the larval stage.

(iii) EFFECT OF RAISED TEMPERATURE ON PUPÆ.

As in the above experiment batches of 10 pupæ were exposed at a time. Pupæ employed were 24 to 48 hours old. Referring again to Chart I, it will be seen that below 38°C. pupæ do not seem to be affected by heat; between this temperature and 40°C. a varying number did not survive an exposure of one hour. Temperature of above 40°C. killed all the pupæ. Below 39°C. over 50 per cent survived, while above 39°C. over 50 per cent died. Between 38°C. and 39°C. they became inert, and above 39°C. they looked apparently dead, though during the next 24 hours a few survived. Pupæ exposed to a nearly fatal temperature never sank to the bottom, but remained quiescent near the top. And even when they were dead they generally floated. That they turned a dark colour on dying has been mentioned above.

* 'Some effect':—Sluggish but showed signs of life without outside stimulation.

† 'Inert':—Sluggish but showed signs of life on outside stimulation.

‡ 'Apparently dead':—Showed no signs of life whatsoever.

The limiting temperature which larvæ and pupæ survived for one hour was fairly well defined. A range of 2°C. between the temperature which gave 100 per cent fatality and at which they all lived, is in conformity with the

CHART I.

Results of one-hour exposures to various temperatures and atmospheric humidities of all stages of the mosquito cycle.

°C.							°F.
40.5	+		+				
40.0	+	+	+				104.0
	+		•				
39.5	○		•	+	+	+	
39.0		+	•	+	+	+	102.2
	○		•	+	+	+	
38.5			•	+	+	•	
38.0	○	+	•	+	+	•	100.4
		+				•	
37.5			○	+	+	•	
		•	○	+	•	•	
37.0	○	•	○	•	•	•	98.6
		•	○	•	○	○	
36.5		•		•	○	○	
36.0		•	○				96.8
		○					
35.5				•	○	○	
35.0							95.0
34.5		○	○				
34.0				•	○	○	93.2
°C.				0	30	60	°F.
				Relative Humidity (per cent.)			
	Eggs.	Larvæ	Pupæ	Adults.			

Signs:-
 + all died; ○ all survived; • some died and some survived.

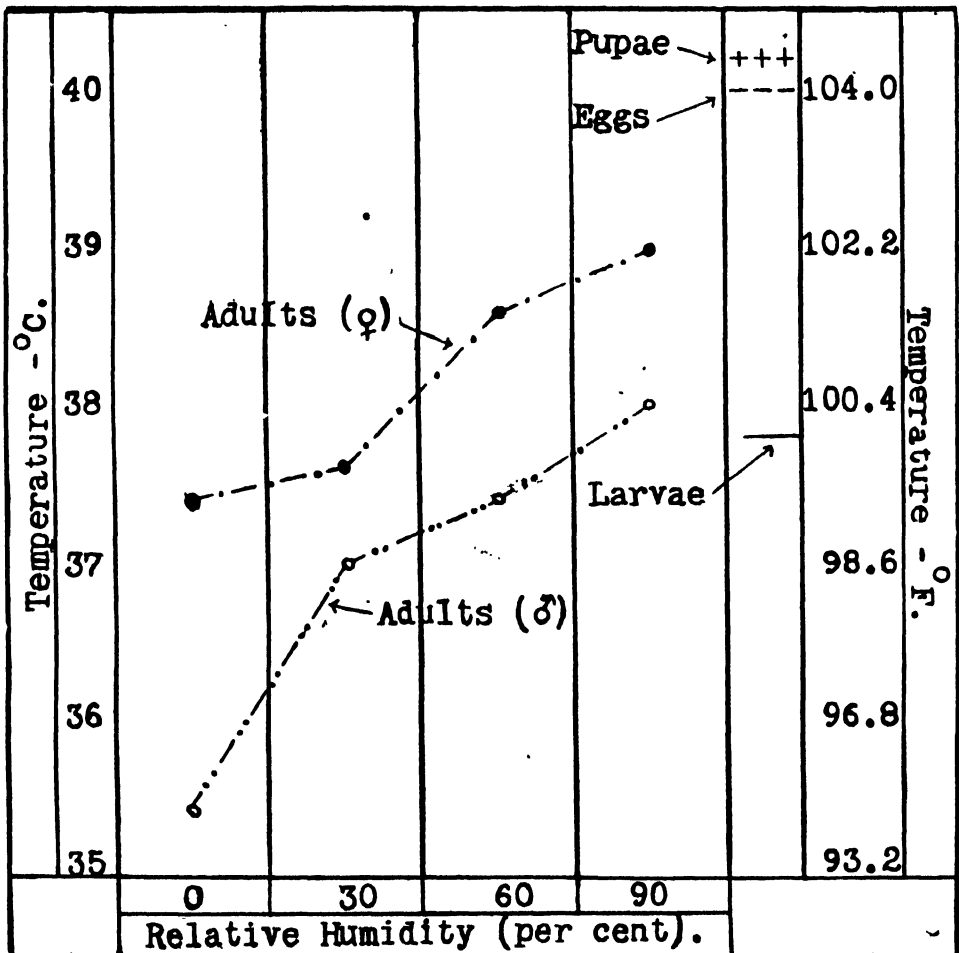
experiments of other workers conducted with other insects (Mellanby, 1932). That pupæ can withstand higher temperatures than larvæ, supports the observations of Macfie (1920) on *Aedes argenteus*.

(iv) EFFECT OF RAISED TEMPERATURE ON ADULTS.

Adults used in these experiments were fed on fruit and water, and were 2 to 10 days old. Mosquitoes, which were flying 24 hours after being exposed, were considered as having survived. Consulting Chart I, it will be seen that the upper limiting range of temperature in this case is modified by another factor, *viz.*, humidity, as these insects live in air. Under the most advantageous conditions, *i.e.*, 90 per cent humidity, it is 39°C., and under the least advantageous conditions, *i.e.*, 0 per cent humidity, it is 37.4°C. on an exposure of one hour. Chart I shows plainly that humidity has considerable effect on mortality at sub-lethal temperatures. This is clear whether we take the upper temperature at which all insects die, or the lower limit at which none dies. It will be observed from Chart II that the lethal limits of temperatures at various

CHART II.

Comparative ranges of lethal temperatures for eggs, larvæ, pupæ and adults.
(One-hour exposures.)



controlled humidities are about a degree or so lower for males than females. With a view to finding out whether females gorged with blood can withstand a higher range of lethal temperatures, experiments were conducted in separate and combined sets. It was found that the results in gorged and non-gorged females were identical.

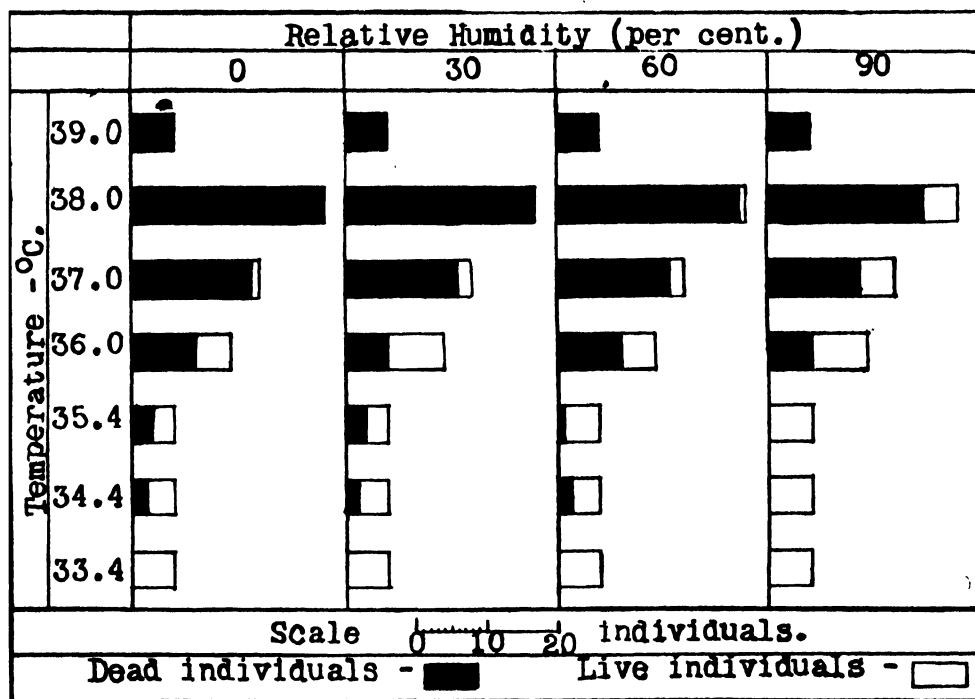
Comparative ranges of lethal temperatures for eggs, larvæ, pupæ and adults are shown in Chart II. The bottom figures of 0, 30, 60 and 90 per cent humidity apply to adults which live in air, and *not* to eggs, larvæ, or pupæ which have an aquatic existence.

(v) INFLUENCE OF HUMIDITY ON THERMAL DEATH POINT OF ADULTS.

It has been shown above that the influence of humidity was apparent between 34°C. and 39°C. even on one hour exposure. Chart III shows this in greater detail.

CHART III.

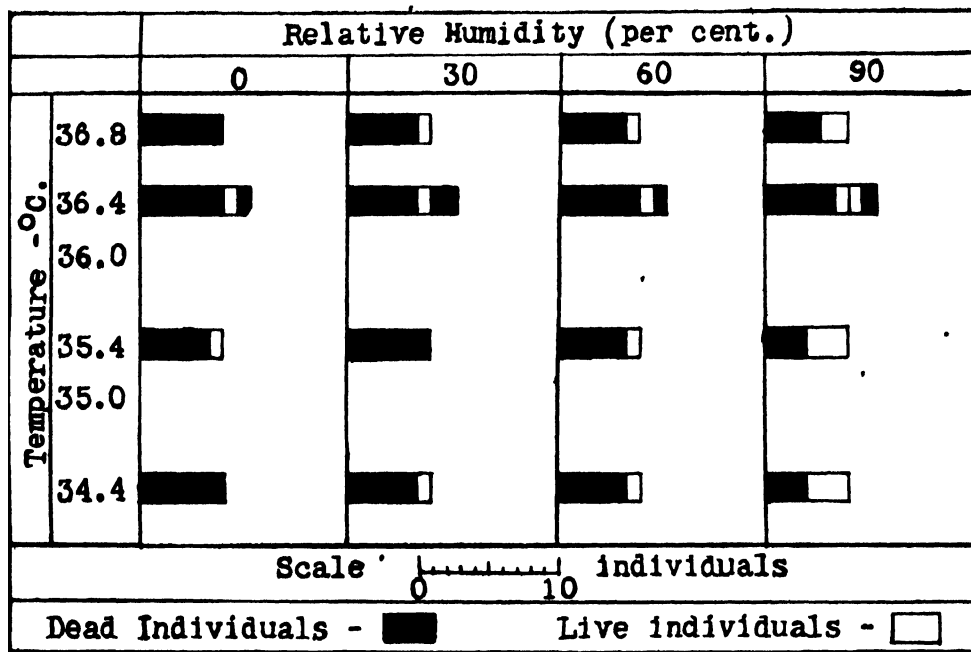
Influence of humidity on the thermal death point of a mixed population (♀ and ♂) of adult mosquitoes.
(One-hour exposures.)



It is interesting to see (*vide* Charts IV and V) that the death point for three and six hours is only a little different from that of one-hour exposures. If these experiments be further analysed, it becomes apparent that humidity

CHART IV.

Influence of humidity on the thermal death point of a mixed population (♀ and ♂) of adult mosquitoes.
(Three-hour exposures.)



is an absolute deciding factor over a narrow range of about 2 degrees between 34°C. and 36°C. Further it will be seen that this mosquito belongs to that group of insects which lose so much water in dry air that the temperature which is fatal to it is lower in dry air than in moist. In other words it cannot conserve water and dies of desiccation. It comes in division two of the classification of Buxton (1933)*, but one must remember that humidity is as significant on one hour exposure as it is on six hours.

(vi) STUDIES IN PHYSIOLOGY—THE WATER ECONOMY OF BOTH SEXES.

It has been shown above that this mosquito survives a rather higher temperature in moister than in drier air for exposures of 1 to 6 hours. It might

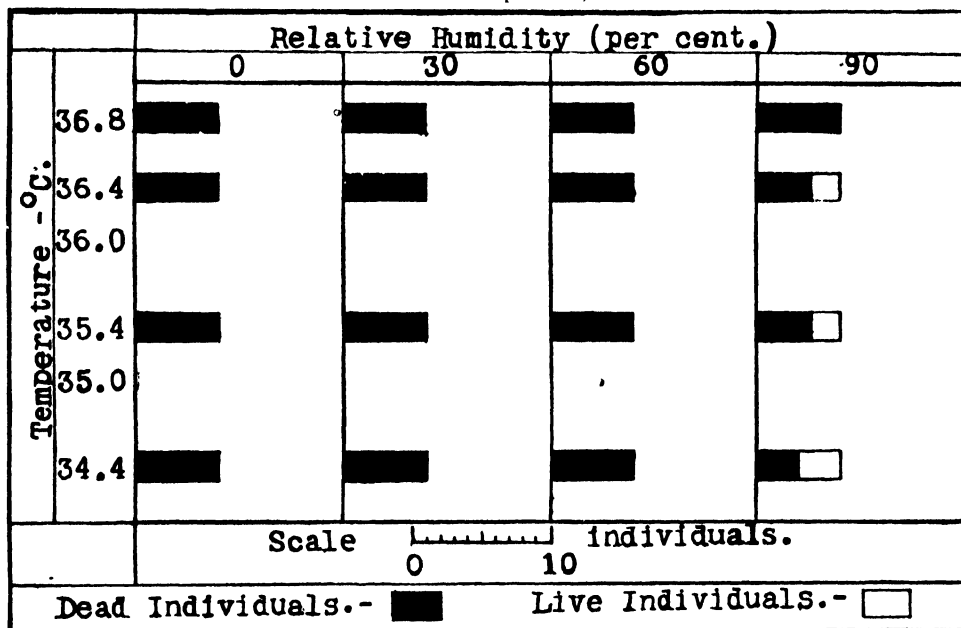
* According to Buxton there are three chief divisions of insects which appear to be distinguishable. In division one, death is due to heat, and occurs at the same temperature irrespective of humidity, whether the insects are exposed for one hour or longer periods. The adults of *X. cheopis* and *C. lectularius* belong to this class. These are called 'savers'. In division two, the insects lose so much water in dry air that the temperature which is fatal to them is lower in dry air than in moist, whether the insects are exposed for one hour or longer periods. The adults of *C. fatigans* belong to this class. These are called 'spenders'. In division three the insects are capable of surviving a higher temperature in dry air than in moist. They regulate their body temperature by evaporation of water, and cool their body in this way. *Tenebrio molitor* belongs to this class. It may however be noted that the larger the insect the more favourably is it situated than the smaller, because it has proportionately less surface, i.e., ratio of its surface to volume is smaller.

be supposed that this is due to evaporation, but it would be more satisfactory to prove that it is so. This I attempted to do by weighing insects after exposure to sub-lethal temperature and controlled humidity, and comparing them with controls. Experiments on both males and females were performed.

CHART V.

Influence of humidity on the thermal death point of a mixed population (♀ and ♂) of adult mosquitoes.

(Six-hour exposures.)



In all cases the individuals were weighed on a torsion balance so as to obtain a statistical measure of reliability of means. Twenty-nine females and nineteen males were weighed from controls, and their mean weight worked out. Twenty-two females and twelve males were experimented with, at each of four relative humidities, and their mean weights calculated. These results are plotted on Chart VI. It will be seen that the loss in weight is consistent. The mean weight of 22 females after exposure to 90 and to 0 per cent humidity is 2.13 and 1.82 mg. respectively, while the mean weight of 29 female controls is 2.43 mg. It is evident that insects after exposure to dry air weighed materially less than the controls or those exposed to 90 per cent humidity. But there is so much variability between the individuals that one cannot tell from the means alone whether the difference is due to the experiment or whether it occurred by chance, if humidity had no effect. One must therefore employ some measure of dispersion, to give an indication of the reliability of the means, and to decide whether the differences between them are significant. Table I gives the salient points of these calculations. It will be noticed that there

CHART VI.

Mean weight of adult mosquitoes after exposure for one hour at different humidities
(Temperature 34°C.)

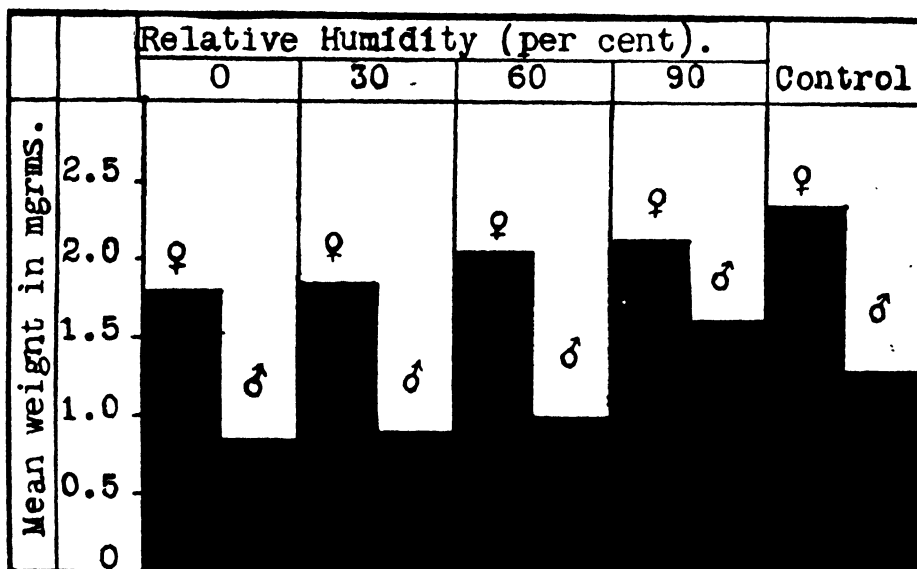


TABLE I.

(A)

	Control.	0 per cent humidity.
Mean	.. 2'343	1'85
Standard error	.. 0'0885	0'124
Standard error of difference	—0'155.	
Difference between means	—0'493.	
Result	:—Difference between means ∇ standard error of difference $\times 2$.	
Conclusion	:—Definitely significant.	

(B)

	Control.	90 per cent humidity.
Mean	.. 2'343	2'130
Standard error	.. 0'085	0'097
Standard error of difference	—0'131.	
Difference between means	—0'213.	
Result	:—Difference between means \angle standard error of difference $\times 2$.	
Conclusion	:—Not definitely significant.	

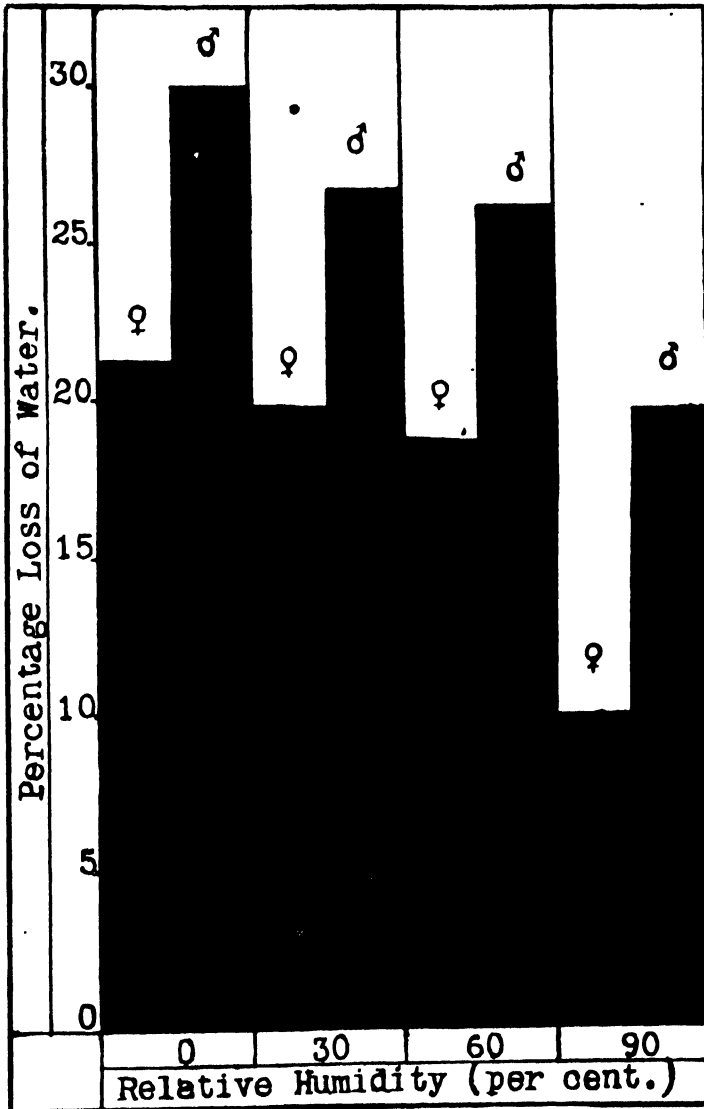
(C)

	90 per cent humidity.	0 per cent humidity.
Mean	.. 2'13	1'85
Standard error	.. 0'097	0'127
Standard error of difference	—0'16.	
Difference between means	—0'28.	
Result	:—Difference between means \angle standard error of difference $\times 2$.	
Conclusion	:—Not definitely significant.	

is statistical support for asserting that there was greater loss of weight in mosquitoes exposed at 0 per cent humidity when compared with controls. But on the comparison of the figures of control with 90 per cent humidity, or

CHART VII."

Percentage loss of water in adult mosquitoes after exposure of one hour at different humidities.
(Temperature 34.4°C.)



90 per cent with 0 per cent, the difference between means is found to be less than twice its standard error. The difference is therefore probably, but not

certainly, significant. If humidity had no effect and the whole thing was a chance, one would get these figures once in about 15 times.

The conclusion not being definitely significant, it was decided to weigh the same individual mosquitoes before and after exposure to different humidities for one hour at 34.4°C. This was a tedious process, because, firstly, the mosquitoes had got to be very lightly chloroformed. It was discovered that mosquitoes which were wriggling were best fitted for experiment, and therefore just enough chloroform had to be given to render them easy to handle. It was important to see that no mosquito escaped, which was worse than its death, as it not only ceased to be useful for experiment but evoked protests from my colleagues. Secondly, the experiments could only be carried out with individuals separately, and thus took considerable time. Finally, it was considered

TABLE II.

90 per cent humidity. Percentage of loss in weight of individuals.		0 per cent humidity. Percentage of loss in weight of individuals.	
	6.19		34.17
	10.77		18.85
	13.22		7.33
	13.86		15.40
	11.08		31.19
	1.78		32.86
	35.06		18.52
	20.29		35.49
	22.22		27.95
	134.47		257.31
Mean	.. 14.94	Mean	.. 25.73

Difference between means = 10.79.

Mean of squares—309.0808	751.392
Square of mean—223.2036.	662.032
Difference—85.8872	89.360
Standard deviation— $\sqrt{85.8872} = 9.2671$	$\sqrt{89.360} = 9.453$
Standard error— $\frac{9.2671}{3^*} = 3.089$	$\frac{9.453}{3^{\dagger}} = 2.986$
Standard error of difference— $\sqrt{3.089^2 + 2.986^2}$	
$= \sqrt{9.542 + 8.916}$	
$= \sqrt{18.46} = 4.263$	

Therefore difference between means, viz., 10.79 \geq standard error of difference, viz., 4.263 \times 2.

Conclusion :—Definitely significant.

* Because there are 9 individuals, and square root of 9 is 3.

† Because there are 10 individuals, and square root of 10 is 3.162.

desirable to experiment with the weighed mosquitoes as soon as possible after they recovered, as otherwise after 6 hours there occurred some difference in weight which vitiated the results. In all, 39 individuals were thus experimented with, and the result is plotted on Chart VII. The results are definitely significant, and leave no doubt about loss of water, not only from a comparison of the means but also from statistical data. These figures are worked out at length in Table II by way of example. The difference of means between 90 per cent and 0 per cent humidities is greater than twice the standard error of difference. It will have been further observed that males lost proportionately more weight than females. This fact, considered along with the death of males at lower temperature and more favourable humidity as compared with females, justifies the conclusion that males probably cannot conserve water as well as females.

Mellanby (1932), experimenting with a number of insects, concluded that in one-hour exposures humidity had no effect on the thermal death point of small insects. His experiments were conducted with insects which had no aquatic existence in their cycle of development, and that may have been the reason why this mosquito behaved differently.

ACKNOWLEDGMENTS.

My most grateful thanks are due to Professor P. A. Buxton of London University, whose guidance during the work, and criticism of this paper, have been very valuable. Thanks are also due to Dr. K. Mellanby who gave valuable suggestions from time to time and verified statistical calculations. I am indebted to Dr. V. B. Wigglesworth for helping me when Professor Buxton was away.

Finally, I have to thank General Sir John Megaw who has kept my interest alive in research, and Colonel Sir S. R. Christophers who arranged for me to work in these laboratories.

APPENDIX.

Table relating to the specific gravity of mixture of sulphuric acid and water to the relative humidity of air over the mixture.

Relative humidity. (Per cent.)	Acid. c.c.	Water. c.c.	Specific gravity.
10	456	400	1.54
20	386	500	1.485
30	374	600	1.41
40	294	600	1.38
50	280	700	1.33
60	204	600	1.295
70	189.7	700	1.25
75	159	600	1.22
80	160	800	1.19
85	112	800	1.15
90	88	800	1.25
95	70	874	1.08

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THE RELATIVE CLINICAL EFFICACY OF TOTAQUINA AND QUININE.

BY

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[12th January, 1935.]

TOTAQUINA is a mixture of cinchona alkaloids prepared according to the formula of the Malaria Commission of the League of Nations (1934). It must contain at least 70 per cent of crystallisable alkaloids and 15 per cent of the total weight must be quinine; not more than 20 per cent may be amorphous alkaloid. It has been introduced as the result of a search for a drug suitable for the treatment of the vast malarious populations of the world which are too poor to buy quinine. As such, it must approach quinine in efficiency and safety, while being considerably lower in cost.

The formula allows for the preparation of Totaquina in two ways. Type I is prepared by the precipitation of all the alkaloids of cinchona bark; type II is obtained from the alkaloids left after the removal of quinine, by adding to the residue enough quinine to bring the final mixture up to standard. It is intended that type I shall be prepared from trees such as *Cinchona succirubra* and *robusta*, which can be grown with comparative ease in many parts of the world, and which give a satisfactory yield of total alkaloids. Type II is suitable for production from *C. ledgeriana*. This species is richer in quinine, and therefore more profitable commercially, but has a more restricted area of growth. It is expected that Totaquina will be cheaper than quinine because all the alkaloids of the bark are used, and because it can be prepared from trees which will grow in many countries, thus evading the present restriction.

The Malaria Commission of the League of Nations, in making arrangements for trials of Totaquina in many parts of the world, laid down the experimental methods which it considered desirable. We have endeavoured to adhere to these recommendations as closely as local conditions allowed, in order that our results might be comparable with those of workers in other countries.

EXPERIMENTAL METHOD RECOMMENDED BY THE MALARIA COMMISSION OF THE LEAGUE OF NATIONS.

The drugs are to be judged by their immediate therapeutic efficacy when given to patients who are in hospital and whose blood contains parasites at the time of treatment.

(a) Give each drug in tablet form by the mouth under medical supervision; the dose for 70 kg. of body weight to be 0·6 gm. for benign tertian, and 1·2 gm. for malignant tertian or quartan.

(b) Treat only those who have asexual parasites circulating in the blood. Give one dose daily, and make a blood film at the same time.

(c) Give the drug and make blood films for five days.

(d) Record toxic symptoms.

(e) The criterion will be the time required for the disappearance of asexual forms. The blood examinations should be carried out by the same technique and the same observer throughout, by counting one hundred fields of a thin film (*frottis*).

(f) The experiment should be made on groups of fifty patients for each drug, without preliminary selection of the patients.

(g)

(h) It is desirable to state whether the case is a first attack, a relapse or a re-infection, and previous treatment should be recorded.

TYPE OF POPULATION UNDER OBSERVATION.

The patients described in this report were male prisoners at the Central Jail and the Borstal Institution of Lahore. At the Borstal Institution, only those patients are included who were sixteen years of age or older, so that their degree of immunity is approximately the same as that of the adults in the Central Jail. The average weight of prisoners was 121·5 pounds; 84·5 per cent of the weights fell between 100 and 139 pounds. Work at the Central Jail was carried on from 17th May to 5th December, and at the Borstal Institution from 6th June to 8th September, 1934.

These men have been exposed to both endemic and periodical epidemic malaria all their lives. It is unlikely that any of our patients was suffering from his first attack, but a distinction between relapses and re-infections could not be made.

The patients were seen by us as soon as they complained of fever to the Jail Medical Officer. Many of them asserted that they had already suffered from fever for several days before reporting to hospital; this may in part account for the rapid response of the infections to cinchona alkaloids. They received no prophylactic quinine, and every effort was made to ensure that they obtained no anti-malarial drug before they were sent to us. We believe that if any of our patients did receive such drugs, it was a very rare occurrence. To confirm this, the urines of a number of new patients, who had

not yet been given cinchona alkaloids by us, were tested by Tanret's reagent. The reaction for quinine was given only by one man, who was not diagnosed as a case of malaria. When the patients left our ward, however, the treatment was completed by a short course of quinine. .

TECHNIQUE.

The blood of all men complaining of fever was examined by thick and thin films. When a diagnosis of malaria was made, the patient was given two grains of calomel, a second thick film of his blood was made, and the parasites were counted by the fowl cell method of Sinton (1924). He was then given the appropriate specific drug in tablet form in the prescribed dosage. Quinine sulphate and Totaquina, types I and II, were given to alternate cases of benign tertian, and similarly to alternate cases of malignant tertian malaria. The patient was examined clinically for signs of intercurrent disease. Temperatures were taken at about 8 a.m. and 5 p.m. A magnesium sulphate mixture was given every morning to all men under treatment, and calomel was repeated when necessary. No other drug was taken.

Every morning the parasites in the patient's blood were counted, and a dose of the specific drug was given shortly afterwards. One dose only was administered each day. This continued until the blood was free from parasites for at least two consecutive days, and there had occurred at least one day free from fever, which was not followed by a subsequent recurrence of fever.

In endeavouring to estimate the frequency of toxic symptoms we avoided leading questions, merely putting to each man the daily question, 'How do you feel?' and recording his answer.

The quinine and both types of Totaquina were provided by the Madras Government Cinchona Department. The composition, as given by them, is shown below :—

			Type I.	Type II.
			Per cent.	Per cent.
Quinine 32	19
Quinidine 1	4
Cinchonine 11	20
Cinchonidine 30	26
Amorphous alkaloid 15	19

The total crystallisable alkaloids in type I are 74 per cent of the mixture, and in type II are 69 per cent. This corresponds roughly to the amount of alkaloid in quinine sulphate, which is 73.5 per cent (Martindale and Westcott, 1924). The tablets were of moderate size, each containing 0.25 gm. of the alkaloids. A dose consisted of one or more whole tablets, together with one tablet which was cut down to a size estimated to complete the correct total dose. This method is rather rough, but on account of the size of the tablets and the small amount of alkaloid in each, we do not think that the error was serious.

It will be seen that the above methods are those proposed by the Malaria Commission of the League of Nations with three exceptions. Firstly, the parasites were counted by the fowl cell method. Secondly, it was impossible to distinguish between first infections, relapses and re-infections, or to record previous occasions on which the patient had been treated for malaria. Thirdly, the treatment was stopped when the patient had been free from parasites for two days and from fever for at least one day.

CONTROL EXPERIMENT.

All the men probably possessed a considerable degree of immunity to malaria, and recovered quickly under treatment with cinchona alkaloids. It appeared possible that they might have recovered nearly as quickly with simple rest in bed and without specific treatment, a state of affairs which would have vitiated any conclusions drawn from figures claiming to give the results of treatment. Accordingly ten benign tertian cases were taken as controls and were put on a course of a simple diaphoretic mixture, without any specific drugs. These alternated with cases taking cinchona alkaloids. In one control case it was necessary to terminate the attack on the second day by giving quinine; this man is not included in the table of results. In another case, Totaquina type I was given on the fifth day (*see* Table VI).

ABSORPTION OF QUININE AND TOTAQUINA.

The tablets of all three drugs disintegrated easily. When a tablet was placed at the bottom of a test-tube of tap water, it broke up in a few minutes without being shaken.

Every dose of cinchona alkaloids was given personally by one of the authors, who afterwards examined the man's mouth with the aid of a tongue depressor to ensure that it had been swallowed. The men showed no dislike for the tablets.

The administration of calomel and magnesium sulphate was intended to bring the intestines of all patients into a suitable condition for the absorption of the alkaloids.

To confirm the absorption, the urines of a number of cases were tested with Tanret's reagent from one to three hours after the administration of the drug. In all cases they gave the reaction for cinchona alkaloids. In view of these facts it may be assumed that the results of treatment were not influenced by inequalities of absorption.

RESULTS.

The results of treatment and of withholding treatment are shown in Tables I to V. In calculating the means for the time of disappearance of parasites, it has been assumed that they disappeared mid-way between the last positive and the first negative film. Thus, if a patient showed parasites on the day of diagnosis, but was free on the next day, he is shown in class 0.1 day, and is considered to have lost his parasites in 0.5 day. In dealing with fever of the malarial type this assumption cannot be made, and the means are

therefore calculated from the whole numbers. Thus, a patient who suffered from fever at 8 a.m. or 5 p.m. on the first and second days only is shown as having fever for two days. When a man had a day free from fever, followed by one on which he had a rise of temperature, both days are recorded as febrile. Only temperatures over 99°F. (37.2°C.) are recorded as fever.

For convenience of comparison, the cases are also shown in Table V arranged according to the method adopted by Pampana in Table 5 of the report of the Health Organisation of the League of Nations (1934).

The number of cases of benign tertian malaria treated was 210 and of malignant tertian was 158. There was one mixed infection. No instance of quartan was observed. A few other cases were diagnosed, which are not included in the results for various reasons which made them unsuitable for the purpose.

The significance of the results has been examined statistically by calculating the values of t and P for the differences of the means (Fisher, 1934). The standard errors of the differences are also shown. In some cases the figures are rather small for the latter method, but it will be seen that it is in general agreement with the results of the former method.

TABLE I.

Persistence of fever and of asexual forms of the benign tertian parasite in control cases taking no specific drug, and in cases treated by cinchona alkaloids at the same time.*

Days.	PARASITES.		Days.	FEVER.	
	Control.	Cinchona.		Control.	Cinchona.
0-1 ..	2	4	1	1	5
1-2 ..	0	7	2	5	3
2-3 ..	3	1	3	2	2
3-4 ..	1	0	4	0	0
4-5 ..	2	0	5	0	0
4-5+ ..	1	0	5+	1	0
Number of cases	9	12	..	9	10
„ afebrile	0	2
Mean in days ..	over 2.83	1.25		over 2.44	1.70
Difference of means.	1.58			0.74	
Value of t ..	3.178			1.644	
Value of P ..	below 0.01			0.1 to 0.2	
	Significant.			Not significant.	

*The patient shown in classes 4-5+ and 5+ received Totaquina on the fifth day. The protocols of his case are presented as Table VI in an appendix.

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TABLE II.

The cases arranged according to the period of persistence of asexual parasites in the peripheral blood as judged by examination of a thick film.

Days.	BENIGN TERTIAN.			MALIGNANT TERTIAN.		
	Totaquina I.	Quinine.	Totaquina II.	Totaquina I.	Quinine.	Totaquina II.
0-1	26	33	36	23	20	29
1-2	38	35	31	20	27	20
2-3	5	0	4	9	6	1
3-4	0	1	1	1	1	1
Number of cases ..	69	69	72	53	54	51
Mean in days ..	1.20	1.05	1.08	1.27	1.28	0.99
Difference of means	0.15	0.03		0.01	0.29	
Standard error of difference.	± 0.100	± 0.105		± 0.146	± 0.132	
Value of <i>t</i> ..	1.487	0.284		0.068	2.173	
Value of <i>P</i> ..	0.2 to 0.1	0.8 to 0.7		over 0.9	0.05 to 0.02	
	Not significant. ^o	Not significant.		Not significant.	Significant.	

TABLE III.

The cases arranged according to the number of days after the beginning of treatment before they became free from fever (over 99°F.).

Days.	BENIGN TERTIAN.			MALIGNANT TERTIAN.		
	Totaquina I.	Quinine.	Totaquina II.	Totaquina I.	Quinine.	Totaquina II.
1 ..	21	22	24	14	13	9
2 ..	23	17	10	20	21	15
3 ..	3	2	1	6	8	7
4 ..	1	0	1	2	2	0
Number of cases ..	48	41	36	42	44	31
Mean in days ..	1.67	1.51	1.42	1.90	1.98	1.94
Difference of means	0.16	0.09		0.08	0.04	
Standard error of difference.	± 0.135	± 0.146		± 0.175	± 0.177	
Value of <i>t</i> ..	1.155	0.613		0.452	0.218	
Value of <i>P</i> ..	0.3 to 0.2	0.6 to 0.5		0.7 to 0.6	0.9 to 0.8	
	Not significant.	Not significant.		Not significant.	Not significant.	

TABLE IV.

The cases arranged according to the method adopted in Table 5 of the report of the Health Organisation of the League of Nations (1934).

			Cases considered.	FEVER.				Cases considered.	SCHIZONTS.			
				Percentage in which the fever disappeared after doses :					Percentage in which schizonts and trophozoites disappeared after doses :			
				1.	2.	3.	4.		1.	2.	3.	4.
<i>P. vivax.</i>												
Quinine	41	54	95	100	100	69	48	99	99	100
Totaquina I	48	44	92	98	100	69	38	93	100	100
Totaquina II	36	67	94	97	100	72	50	93	99	100
<i>P. falciparum.</i>												
Quinine	44	30	77	95	100	54	37	87	98	100
Totaquina I	42	33	81	95	100	53	43	81	98	100
Totaquina II	31	29	77	100	100	51	57	96	98	100

TABLE V.

Incidence of toxic symptoms. Percentages of those who complained of vomiting or vertigo.

VOMITING.				
Days.	Quinine.	Totaquina I.	Totaquina II.	Number of cases for each drug.
1	7.4 per cent	13.2 per cent	15.7 per cent	51—54
2	3.7 " "	15.1 " "	9.8 " "	51—54
3	0	3.8 " "	3.9 " "	51—54
4	0	0	0	34—43

VERTIGO.				
Days.	Quinine.	Totaquina I.	Totaquina II.	Number of cases for each drug.
1	0	0	2 per cent	51—54
2	1.9 per cent	3.8 per cent	3.9 " "	51—54
3	9.3 " "	9.4 " "	2 " "	51—54
4	4.7 " "	5.7 " "	0	35—43

DISCUSSION OF RESULTS.

The material for the experiment consisted of Indian males who were sixteen years of age or older. It is unlikely that any were suffering from their first attacks of malaria, and perhaps many of the benign tertian cases were relapses. Probably all the patients had some degree of immunity, varying with the individual, but the number of cases recorded is large enough to suppress serious errors arising from the individual factor. This also applies to the question of a possible multiplicity of strains of parasite, of which some may have been more amenable to cinchona than others.

The dosage was that recommended by the Malaria Commission of the League of Nations (1934). It was small and calculated to display slight differences in efficacy better than larger doses would have done. Where two drugs differ slightly in their action and are given in massive doses, these slight differences are likely to be masked.

The number of cases in the control experiment recorded in Table I is low, but is sufficient to show that the cinchona alkaloids exerted a definite effect on parasites, though their action on fever is not so well shown. Moreover, the difference between the control and cinchona groups would probably have been greater than that shown in the table, if one of the control patients had not received Totaquina on the fifth morning. Consequently the figures in Tables II and III may be taken as showing the relative actions of the three drugs.

If the criterion of the relative efficacy of quinine and Totaquina is taken as the mean time required for the disappearance of parasites and fever, it will be seen from Tables II and III that, though on the whole the variations are in favour of Totaquina type II, in most cases the differences between the drugs are small and are within the limits of error due to random sampling. According to conventional usage, differences have been considered as 'not significant' when P is greater than 0.05 or the difference of the means is less than twice the standard error of that difference. By this test, the only case which is significant is that in which malignant tertian parasites disappeared more quickly under treatment with Totaquina type II than with quinine. Here P is slightly less than 0.05, and the difference is slightly more than twice its standard error. It is possible that Totaquina type II is more lethal to *P. falciparum*; but on the other hand there is some evidence that chance distributed milder cases to this drug for, among the patients treated by it, fever was recorded in only 31 (61 per cent), while the figures for quinine are 44 (82 per cent) and for Totaquina type I are 42 (79 per cent). It will be seen that Totaquina type II was not superior in banishing fever from those malignant tertian cases which suffered from fever.

The contrary seems to have occurred in the results for benign tertian, where Totaquina type I has been less successful than either of the other two drugs in destroying parasites. Here the figures for febrile cases are for quinine 41 (59 per cent), for Totaquina type I are 48 (70 per cent) and for type II are 36 (50 per cent).

Our conclusion, therefore, is that, under the conditions of this experiment, there was no distinct difference in efficacy between quinine and the two types of Totaquina in clearing the blood of parasites and in subduing fever. As to the relapse rate, we have no data on which to form an opinion.

DISCUSSION OF TOXIC EFFECTS.

The toxicity of the samples was not easy to estimate. The statements of prisoners about subjective symptoms are not always reliable, as they naturally find it easy to remember those which they hope will ensure a longer stay in hospital. It was noticeable that toxic symptoms occurred in epidemics. When one man complained of vertigo, others in the ward would remember that they had been similarly troubled. At another time the prevailing complaint was 'weakness'.

In Table V are shown for the first four days of treatment the percentages of those complaining of vertigo or vomiting. The figures for the fifth and subsequent days are smaller and unsuitable for comparison. Only those are included in the table who had malignant tertian malaria, and so were taking the larger dose of 1.2 gm. for 70 kg. of body weight.

For vertigo the figures show no really striking differences. The incidence increased on the second and third days, and the symptom is therefore probably partly due to the drug.

For vomiting the figures seem to show an advantage in favour of quinine. But as the percentages decrease on the second and subsequent days, the symptom is probably due to the disease more than to the drug.

In view of the unreliability of the complaints on which the records are based, it would be unwise to place much weight on the small differences observed, or to conclude that our figures display any real difference in toxicity between the three drugs. Indeed, it would have been surprising if this small daily dosage had provoked clearly defined toxic symptoms. In this connection it may be noted that Fletcher (League of Nations, 1934), when reviewing the trials of Totaquina conducted under the auspices of the League of Nations, concluded, 'As regards toxicity, the case records contain no cogent evidence that Totaquina is more toxic than quinine in the doses given'.

The above results are in agreement with those which have been obtained in trials in other countries, and have been published by the Health Organisation of the League of Nations (1934). The practical application remains to be considered.

OPTIMUM DOSAGE.

These experiments give some evidence of the optimum dosage for a population having a degree of immunity comparable to that of our patients. Among all our men, only one had urgent symptoms. This was a case of malignant tertian malaria, who became comatose within an hour of taking his first dose of Totaquina type I. It cannot therefore be considered as showing insufficiency of drug or dosage. We suggest that a dose of 1.0 gm. or 15 grains once daily for three or four days would be suitable for the routine treatment of rural populations in the Punjab. This should be large enough to prevent the majority of deaths and to remove clinical symptoms, which is all that is demanded by such populations.

THE POSITION OF TOTAQUINA AS AN ANTI-MALARIAL DRUG.

A drug which is to be distributed to poor malarious populations must be efficient, safe and cheap. The evidence shows that both types of Totaquina

are efficient and safe, but the question of price is more difficult. It would be out of place to discuss it at length in this paper, but the following general considerations may be of interest.

The mixture of cinchona alkaloids known as cinchona febrifuge is cheap because it is a by-product of the manufacture of quinine. If Totaquina type II is produced by adding quinine to a mixture of residual alkaloids, its price will be higher than that of cinchona febrifuge by the cost of the added quinine and the expenses of analysis. The latter might be a serious addition to the cost if spread over a comparatively small bulk of the drug. Thus on a small scale, Totaquina must be more expensive than cinchona febrifuge. But Totaquina was devised as a drug suitable for distribution on a large scale. In this case it will no longer be a by-product, and its price must rise, unless the sale of quinine increases proportionately. It would be necessary to devote special plantations to its production; all the alkaloids of the bark would then be used, and the Totaquina prepared would be type I. It would differ from a cinchona febrifuge produced on the same scale only by the necessary analysis, of which the cost should not be very important when spread over a large bulk of the drug. Thus, on a small scale, Totaquina would probably be considerably more expensive than cinchona febrifuge, while on a large scale its cost would be little more than that of cinchona febrifuge prepared on the same scale.

Totaquina should remain cheaper than quinine. The cost of maintaining a plantation for its production will be the same as if the plantation were intended for quinine. On the other hand, there are two factors which tend to keep its cost below that of quinine. In the first place, the fact that it can be produced from trees which will grow in many countries allows the planter to evade the present control of the market. In the second place, as the total alkaloids are precipitated instead of quinine alone, the yield of drug from each tree will be higher than if quinine alone were separated:

It is evident that the price of Totaquina must lie between that of quinine and that of cinchona febrifuge, approaching one or the other according to the type used and the scale of production. Our conclusion is that it may be substituted for quinine without loss of efficiency in reducing the clinical manifestations of malaria in regions comparable to the Punjab. But in India, where cheapness is essential, cinchona febrifuge cannot easily be dismissed. Many trials of this preparation have been made, the results of which have been summarised by Sinton (1930). They show that cinchona febrifuge approaches quinine in efficacy. Its most evident inferiority to Totaquina lies in the fact that it is not standardised, and so is liable to variation and adulteration. If the cost of the drugs shows much difference, it is doubtful if the advantages of Totaquina outweigh its higher cost, when intended for the treatment of really poor populations. If the cost of the two drugs is approximately the same, Totaquina would probably be preferred.

Another possibility in the use of Totaquina is suggested by Field (1934). A reduction in the cost of synthetic drugs might force quinine producers to lower the price of quinine until it was no longer profitable. In this event, the substitution of Totaquina might enable them again to enter into competition.

APPENDIX.

The following case is of interest as showing the rapid action of cinchona alkaloids in a patient who has been allowed to undergo several paroxysms of malaria.

TABLE VI.

Record of a case of benign tertian malaria who received no specific treatment until the fifth day when he began a course of Totaquina type I.
(N = temperature 98°4' F. or less.)

Days.	Number of asexual forms per c.mm.	Presence of gametocytes.	TEMPERATURE.		Treatment.
			A.M.	P.M.	
1	810	+	N	103·4	Calomel grains 2, diaphoretic mixture.
2	2,490	++	N	100·4	Diaphoretic mixture.
3	1,950	+++	N	104·4	Ditto.
4	3,450	+++	N	105·0	Ditto.
5	1,740	+++	N	103·2	8 a.m. Parasite count. 10 a.m. Totaquina type I. Totaquina, standard dose each morning.
6	0	0	N	N	Ditto.
7	0	0	N	N	Ditto.
8	0	0	N	..	Ditto.

SUMMARY.

1. Adult male prisoners in Lahore who were suffering from malaria were treated with quinine and Totaquina, types I and II, in strict rotation, according to the method recommended by the League of Nations.

2. In benign and malignant tertian malaria there was no distinct difference in efficacy between quinine and the two types of Totaquina in causing the disappearance of parasites and fever.

3. The evidence as to toxicity was not very reliable. It failed to show any significant difference in toxicity between quinine and the two types of Totaquina.

4. All three drugs were well absorbed.

5. A suitable dose for poor malarious populations is suggested.

6. The indications for the choice of Totaquina, quinine or cinchona febrifuge are discussed.

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NOTES ON MALARIA ON THE BARSİ LIGHT RAILWAY (DECCAN).

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[1st October, 1934.]

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I. INTRODUCTION.

THE Barsi Light Railway, which is 202½ miles long and has 41 stations, runs from Miraj (Southern Maratha country) in a north-easterly direction to Latur (Osmanabad District, Hyderabad State). It serves the country lying between the river Krishna ('Kistna') in the south-west and the Manjra in the north-east. After leaving Miraj State, it traverses parts of Sangli State, Jath State and Sholapur District before entering the Osmanabad District of Hyderabad State. It is linked up with the main G. I. P. line from Poona to Sholapur at Kurduvadi station.

The country traversed by the railway is slightly undulating, with elevations ranging from 1,500 to 2,200 feet above sea level, the lowest point being at Pandharpur (1,509 feet), and the highest between Murud and Neoli (2,263 feet). Except for the region surrounding Miraj, the land tends to become flatter, less rocky and covered by a layer of soil as the line proceeds north-eastwards. This does not hold true throughout, however, for there is a belt of hilly country between Pangri and Yedsi stations which has the character of a spur from the Western Ghats. There is also a tract of bare rocky hills terminating 2 miles to the west of Sulgare station, and another region of treeless hills with stony barren slopes and rocky ravines, evidently off-shoots from the Mahadev range, between Dhalgaon and Jath Road stations. Almost everywhere else a good deal of this arid country has been brought under cultivation, and fields, where millets, cotton and oil-seeds are grown in the black soil, alternate with stony waste land and meagre pasturages.

Under the black soil, which is seldom deeper than 2½ feet, there is usually a layer of murum; there are also extensive tracts of murmad soil, with here and there patches of red soil. Many of the nullahs run through the murum, and their beds are made up of boulders, gravel and sand. A few, particularly on the south-western section, run in rocky beds. Various minerals, such as flint, calcite and glauconite, usually encountered with trap formation, are commonly found either in the nullahs, or scattered elsewhere.

The commonest tree is the babul, which thrives almost everywhere. Otherwise higher vegetation is scarce, except at the margins of the larger nullahs and on the hillocks and ravines in the vicinity of Ramling, where a considerable variety of small trees, bushes and creepers is to be found.

No temperature or humidity figures are available for the railway stations. The hottest months are April and May; the maximum temperature may rise to about 117°F. in the latter month at Kurduvadi. The cold season lasts from December to February, the minimum temperature falling to below 60°F. at night. The monsoon usually sets in at the beginning of June, and the return monsoon, which is of short duration, in October or November, sometimes earlier. The rainiest months are usually June or July and September or October. The only available rainfall figures are given in Table I.

TABLE I.
Monthly statement of rainfall. (In inches.)

Years.	Stations.	Jan'y.	Feb'y.	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
1930	Miraj	0-05	3-71	1-63	3-15	0-75	9-48	8-59	0-50	1-20
"	Langarpeth	3-03	2-25	0-56	0-06	11-97	3-34	0-10	0-77
"	Jath Road	0-15	0-40	0-29	13-20	3-28	0-50	..
"	Bamani	0-20	0-40	2-40	0-10	1-55	6-76	6-47	4-45	0-20
"	Pandharpur	1-17	1-82	4-33	2-29	0-65	6-82	3-10	2-90	..
"	Ashti	3-12	2-68	..	9-42	3-10	4-15	..
"	Kurduvadi	0-06	3-13	1-85	0-71	0-23	6-77	5-51	3-23	0-03
"	Barsi Town	0-80	9-35	1-33	1-35	10-71	6-85	1-01	..
"	Kalamb Road	0-06	0-15	..	1-05	0-76	0-38	22-06	7-12	3-39	..
"	Latur	0-10	..	1-15	..	0-45	2-70	2-60	17-85	2-05	1-75	..
1931	Miraj	0-72	0-60	5-52	4-45	2-42	3-37	2-23	3-32	1-21
"	Langarpeth	2-94	0-33	5-20	2-23	0-68	5-81	6-00	3-06	1-18
"	Jath Road	0-80	..	3-75	0-15	..	9-15	1-95	4-70	1-47
"	Bamani	2-17	0-10	6-30	2-10	0-65	4-00	1-10	4-25	0-70
"	Pandharpur	1-24	..	5-10	4-02	0-52	3-63	2-81	2-65	0-76
"	Ashti	0-20	0-85	0-15	9-45	3-60	1-60	6-99	4-49	3-05	0-60
"	Kurduvadi	0-11	..	11-61	2-10	1-57	6-41	5-12	1-55	0-89
"	Barsi Town	0-07	..	11-09	4-98	5-70	7-23	4-08	1-46	0-91
"	Kalamb Road	9-55	12-35	9-45	8-36	4-05	2-65	0-65
"	Latur	6-00	2-30	3-25	5-80	3-10	1-10	0-90

TABLE I—*concl'd.*

Years.	Stations.	Jan'y.	Feby.	March.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
1932	Miraj	0·08	2·12	1·96	0·45	6·73	11·57	1·55	9·42	4·78	..
"	Langarpeth	3·10	0·90	4·55	5·60	1·05	6·00	1·70	..
"	Jath Road	0·70	2·40	4·10	6·55	3·20	3·55	2·75	..
"	Bamani	0·20	..	1·00	2·15	2·25	4·65	1·47	4·20	6·30	..
"	Pandharpur	0·50	0·25	1·20	5·22	4·10	6·26	2·19	5·60	4·89	..
"	Ashti	0·40	..	1·45	4·65	6·20	10·00	2·70	4·00	4·00	..
"	Kurduvadi	0·20	0·85	0·35	2·84	9·41	9·20	3·88	3·79	5·12	..
"	Barsi Town	0·30	0·20	1·38	3·50	7·12	7·85	4·00	6·65	6·10	..
"	Kalamb Road	0·15	0·08	2·65	7·90	6·47	8·05	5·33	4·35	2·95	..
"	Latur	0·14	0·57	5·54	6·69	4·80	5·16	1·53	1·65	..
1933	Miraj	0·18	0·06	3·55	2·25	0·66	8·65	1·08	8·32	1·82	1·03
"	Langarpeth	..	0·10	..	0·60	4·40	1·40	0·70	2·90	2·75	7·10	1·25	1·00
"	Jath Road	3·38	5·04	2·45	3·67	3·81	3·72	10·60	2·40	..
"	Bamani	..	0·10	..	0·30	1·87	0·90	0·55	4·54	2·35	4·03	0·90	3·24
"	Pandharpur	1·12	2·13	3·11	..	3·45	2·11	4·84	1·13	0·18
"	Ashti	1·80	2·70	0·45	0·73	3·57	3·75	4·11	1·04	..
"	Kurduvadi	0·07	0·24	..	3·46	0·90	5·02	0·99	3·80	7·32	4·45	1·53	1·02
"	Barsi Town	..	0·25	..	1·60	2·23	1·57	4·85	3·58	9·05	3·98	0·85	0·48
"	Kalamb Road	0·10	0·55	..	1·35	2·00	4·40	2·25	6·85	8·28	2·05	2·50	0·70
"	Latur ..	0·10	1·10	0·05	2·15	5·17	10·55	5·50	8·98	8·55	1·75	2·85	0·70

The prevailing wind is from the south-west from the beginning of June to September or October. From October till the end of the hot season north-easterly winds often prevail, but are not constant.

II. ANOPHELINE MOSQUITOES.*

1. BREEDING PLACES OF ANOPHELINES.

The chief actual and potential breeding places of Anophelines are as follows :—

(1) Pools remaining in nullahs, if the rains have been heavy. These vary greatly in number and extent from year to year. They are favourite breeding places of *A. culicifacies*.

(2) Pools in river beds, not connected directly with the main stream. Larvæ have not been found at the margins of the main streams in the large river beds (Krishna, Bhima, Sina, Manjra, Man).

(3) Pools remaining in cart tracks after rain, or when water has been drained off from the fields into them.

(4) Seepages in connection with reservoirs. A certain amount of Anopheline breeding is found in these, but not in the reservoirs themselves except those at Pandharpur and Ashti.

(5) Borrow-pits along the course of the railway line.

(6) Wells. Anopheline breeding is unimportant in these, probably on account of the presence of fish and various insect enemies. No breeding has been noted in the irrigation channels from the wells, where the current is swift and intermittent, and seldom stagnates.

(7) Dhobis' tanks at Latur, Barsi Town and Miraj. No Anopheline breeding has been found in these.

(8) Temple tanks in villages have similarly yielded no Anopheline larvæ in any considerable numbers.

(9) Cracks in the black cotton soil have never yielded larvæ, and in pools formed in depressions in this soil only the larvæ of *A. subpictus* have been found.

2. ROUTINE OF SURVEY.

The present survey was planned to include monthly catches of larvæ within at least a one to two mile radius from each station, and it should have been completed in October 1934. Unfortunately it had to be discontinued at the end of February 1934, with the result that data are lacking regarding 78 out of the contemplated 492 seasonal station visits.

Owing to lack of staff it was not possible to give proper attention to the breeding out of the larvæ captured, so that the mortality amongst these was enormous. Each figure of hatched mosquitoes given should probably be multiplied by about eight in order to obtain an approximately correct idea

*This part of these notes has been considerably abridged, as many of the detailed observations on mosquito distribution, etc., were of local interest only. They have been summarised in Tables II and III. The original paper, with its local data and maps of the distribution of the various species of Anophelines, is available for reference in the library of the Malaria Survey of India, Kasauli.—(Editor).

TABLE

Stations.	<i>A. culicifacies.</i>		<i>A. subpictus.</i>		<i>A. stephensi.</i>		<i>A. theobaldi.</i>		<i>A. annularis.</i>		<i>A. moghulensis.</i>		<i>A. turkhudi.</i>		<i>A. fuscistilis.</i>	
	L	A	L	A	L	A	L	A	L	A	L	A	L	A	L	A
Miraj ..	125	..	202	..	19	..	3
Bolwad ..	140	..	48	..	39	..	3	1
Arag ..	179	..	23	..	58	..	5	..	1	..	3	..	13	..	1	..
Athni Road ..	198	..	31	..	160	1	..	12	..	1	..
Sulgare ..	142	..	11	..	42	3	..	1	..
Dhulgaon ..	277	..	80	4	83	..	1	8	..	6	..
Kavathe Mah- ankal.	196	..	13	..	28	..	7	3	..	10	..	13	..
Langarpeth ..	326	4	2	..	44	..	6	11	..	7	..	7	..
Dhalgaon ..	307	..	22	..	96	..	25	1	..	22
Gulvanchi ..	168	..	71	..	65	..	1	1
Jath Road ..	356	8	16	3	89	2	2	4	..	1
Javla ..	140	..	6	..	106	3
Wasud ..	60	2	7	1	6	10
Sangola ..	248	..	35	..	109	43
Bamani ..	155	..	4	..	76	..	1	6
Bohali ..	153	..	6	..	18	2	..	2	..
Pandharpur ..	311	241	238	108	16	17	336	59	1	2	..	4
Babhulgaon ..	104	..	46	..	32	1	10	..	8
Asthi ..	365	4	123	1	35	..	2	..	126
Modlimb ..	234	..	18	..	15	..	1
Padsali ..	142	..	58	..	7	..	3	18	2	..
Laul ..	293	..	103	..	33	..	10
Kurduvadi ..	197	84	937	11	10	4	4	5	2
Chink Hill ..	205	..	37	..	33	..	56	1	4	..
Mahisgaon ..	371	..	110	..	75	..	20	8
Shendri ..	206	..	18	..	60	..	2
Uplai ..	92	..	20	..	90	..	4	1
Barsi Town ..	198	..	70	..	40	..	4
Kuslamb ..	99	..	5	..	12	..	13	..	3	..	1	14	..
Pangri ..	166	..	4	..	35	..	27	51	..	11
Ramling ..	114	..	6	..	3	..	95	..	1	..	109	..	11	..	3	..
Yedsi ..	105	..	1	..	7	..	10	..	1	..	10	..	2	1	3	..
Kalamb Road ..	237	..	22	..	47	..	89	2	7	..
Dhoki ..	160	..	17	..	170	..	25	2
Thair ..	143	..	3	..	35	..	45	1	..	2
Palsap ..	165	..	8	..	68	..	30	2	..	2	..
Murud ..	108	..	32	..	19	..	20	..	1	..	10	3	..
Neoli ..	174	..	9	..	26	..	4	..	1	3	..	12	..
Owsa Road ..	142	..	9	..	34	..	3	2	..	11
Hangul ..	129	..	3	..	19	..	16	1	3	..
Latur ..	146	3	58	14	44	..	11	2	..	7	..

'L' stands for 'Bred from larvae'.

II.

<i>A. pallidus.</i>		<i>A. hyrcanus</i> var. <i>niger-rinus.</i>		<i>A. barbrostris.</i>		<i>A. maculatus.</i>		<i>A. tessellatus.</i>		<i>A. jamesi.</i>		<i>A. splendidus.</i>		<i>A. philippinensis.</i>		<i>A. karwari.</i>		<i>A. vagus.</i>
L	A	L	A	L	A	L	A	L	A	L	A	L	A	L	A	L	A	L
..
..
..	1	1
5	1
..	..	2
..
..
..
2
1	..	1
1	..	1
1	..	41	..	9	1	..	1	1
57	2	10	2	29
5
13	..	1
..
..	1
..	5	..	2	..	1	1
..	..	6	1
..	2
..
..	4
..	1
..	1	1
..
..	4	..	3	1	..	1
..
..
..	..	2	..	7	..	1	1
..
..	..	1
..	..	1	..	1	..	4
1	3

and 'A' for 'Adults caught'.

TABLE

Species.	JANUARY.		FEBRUARY.		MARCH.		APRIL.		MAY.	
	L	A	L	A	L	A	L	A	L	A
<i>A. culicifacies</i>	1,074	22	679	4	290	..	340	..	416	..
<i>A. subpictus</i>	176	3	186	6	147	5	208	..	135	..
<i>A. stephensi</i>	627	1	400	1	68	..	84	..	34	..
<i>A. theobaldi</i>	117	..	67	..	3	..	6	..	2	..
<i>A. annularis</i>	263	30	3	5	96	15	48	3	12	..
<i>A. moghulensis</i> ..	29	..	45	..	2
<i>A. turkhudi</i>	80	1	31	..	26	..	1
<i>A. fluviatilis</i>	24	1	1	3	..
<i>A. pallidus</i>	13	..	4	..	11	..	7
<i>A. hyrcanus</i> var. <i>nigerrimus</i>
<i>A. barbirostris</i> ..	8	1
<i>A. maculatus</i>	2	..	2	..	6
<i>A. tessellatus</i>
<i>A. jamesi</i>
<i>A. splendidus</i>	2
<i>A. philippinensis</i>
<i>A. karwari</i>
<i>A. vagus</i>

'L' stands for 'Bred from larvæ'.

regarding the larval catches, at any rate as far as the commoner species are concerned.

Adult mosquitoes were not searched for systematically, and the few captures mentioned are hardly representative, with the possible exception of the Pandharpur railway colony, where they were looked for more consistently at the various seasons. No dissections were carried out.

The records of larval and adult catches at each station are shown in Table II, and the catches of the various species by months are shown in Table III.

III.

JUNE.		JULY.		AUGUST.		SEPTEMBER.		OCTOBER.		NOVEMBER.		DECEMBER.	
L	A	L	A	L	A	L	A	L	A	L	A	L	A
405	2	695	9	511	41	593	47	503	60	952	31	1,308	130
197	6	91	3	139	18	75	43	246	34	524	13	402	11
25	3	72	1	31	..	26	3	19	1	159	4	458	10
..	38	..	95	..	230	..
..	1	1	11	..	17	1	6	..	21	4
..	..	49	1	..	11	..	12	..	80	..
..	..	23	..	4	..	7	11	..	15	7
..	10	..	3	..	6	..	11	..	33	3
..	23	..	23	..	5	2
..	2	..	8	..	55	1
..	5	17	..
..	8	..
..	1	2	1	..
..	1	..	1	..	1	..
..
..	2
..	1
..	30

and 'A' for 'Adults caught'.

My sincere thanks are due to the officers of the Malaria Survey of India for kindly identifying mosquitoes sent to them, and for arranging for the identification of aquatic plants by the Superintendent, Royal Botanic Gardens, Calcutta, and to Dr. Hora of the Zoological Survey of India for kindly identifying certain fish forwarded by the Secretary, Bombay Natural History Society.

3. NOTES ON BREEDING PLACES, DISTRIBUTION AND BIONOMICS.

(1) *A. culicifacies*.

The chief breeding places of this species, which is the commonest *Anopheline* in the area, are pools in nullahs, especially when these are shallow.

The presence of vegetation is not a necessary condition. The larvæ have not been found in water where there is a current unless this be slow, and then they are found mainly in backwaters, where they are sheltered by grass or aquatic plants. In waters where fish (e.g., *Barilius*) are found, the larvæ are scarce or absent.

No seasonal prevalence of breeding in the nullahs has been observed, other than that determined by the amount of water remaining in them. Larvæ have been found in large numbers during both the hottest and coldest seasons of the year, and during the height of the monsoon. In the last case they are, however, washed away by heavy rain, and are only found during breaks in the monsoon.

Other breeding places are shallow pools and hoof prints at the margins of tanks, wells where the water is rarely disturbed and vegetation is present, and borrow-pits along the course of the line. The species has not been found breeding, as a rule, in dirty water, being essentially a clean water and rural breeder. The following variations were noted in certain specimens identified by the Central Malaria Bureau :—

- (i) Specimens with pale fringe spots opposite all veins except the sixth.
- (ii) Specimens with a pale third vein.
- (iii) Specimens having the inner quarter of the costa without a pale interruption, and with narrow scales on the extreme front of the dorsum of the thorax.
- (iv) Specimens with very faint spots on the costa and no clear light spots on the veins.
- (v) Specimens with a large whitish yellow spot on the tibio-tarsal joint of the hind legs.

(2) *A. subpictus*.

This is the second species as regards frequency, and given suitable collections of water it shows no seasonal prevalence whatever. In its breeding habits it is the least fastidious of all the common species in the area. Its favourite breeding places are borrow-pits, ditches and pools containing muddy or slightly polluted water in the neighbourhood of habitations. It is found breeding also in pools in the nullahs, wells, tanks and water-troughs, but usually in scanty numbers. Its preference for breeding in stagnant pools near dwellings makes it much more of an urban species than any of the other Anophelines of the area. Adults are usually found in quarters at the larger stations, such as Kurduvadi and Pandharpur.

One female of this species, having the distal pale band on the palpi divided by a dark narrow band, was encountered.

(3) *A. stephensi*.

This species comes second in frequency as regards malaria-carrying mosquitoes, although it is far less numerous than *A. culicifacies*, the total number hatched out being only about one-fourth of the latter species. It has been found at all the stations on the line, and during every month in the year, though it is only really numerous during the four cold weather months. It has been

observed repeatedly that the rate of development of the larvæ of *A. stephensi* is considerably slower than those of *A. culicifacies*.

Along the railway, *A. stephensi* is chiefly a nullah-breeding species, and is usually found along with *A. culicifacies* in shallow pools with clear water and scanty or no vegetation. It has been found breeding in wells rather more frequently than *A. culicifacies*, but the larvæ have only occasionally been collected from pools, borrow-pits and ditches. Two specimens were hatched out from the Pandharpur tank, and in a few swamps it has been the only Anopheline larva observed, though always in scanty numbers only. It is not considered likely to play a very important rôle as a malaria carrier on this railway. From the variety of breeding places in which it has been found, however, the writer has received the impression that it is somewhat more adaptable to the various conditions obtaining in the various water collections than is *A. culicifacies*, though as regards seasonal distribution it appears to be more restricted.

The following unusual conditions were observed in two specimens identified at the Central Malaria Bureau as *A. stephensi* :—

(i) A male without speckling on the palpi, and having no scales on the abdominal segments except on the last; and with only a few narrow hair-like scales on the dorsum of the thorax.

(ii) A specimen having the sixth wing vein without a dark spot.

(4) *A. theobaldi*.

A. theobaldi is on the whole a common Anopheline in the rural districts. It has an extensive distribution, although there are seven stations on the line, Pandharpur among them, where it has not been found. It is essentially a nullah breeder, and its larvæ have been found, along with those of *A. culicifacies* and a few *A. stephensi*, in shallow pools of clear water, especially those containing fresh green algæ. It has occasionally also been found breeding in wells and in seepage pools containing clean water. The larvæ have been frequently collected from pools in the ravines near Ramling. Adults have never been captured in quarters, and the species is definitely uncommon where more or less urban conditions prevail, as at the larger stations.

A. theobaldi is not considered to play any part in the transmission of malaria on this railway.

Its seasonal distribution appears to be much more definite than that of *A. stephensi*, showing a prevalence during the cold months of the year. It first appears at the end of the monsoon.

(5) *A. annularis* (*A. fuliginosus*).

A. annularis has a wide distribution, the larvæ being present in scanty numbers in clear water pools in the nullahs throughout the course of the line. It is however pre-eminently a tank breeder, the larvæ being found in large numbers in the tanks at Pandharpur and Ashti. In both these tanks it shows to some extent a seasonal restriction, alternating with *A. culicifacies*. Breeding of *A. annularis* commences at the end of the monsoon and continues throughout the winter; and, during the hottest months of the year, the larvæ have been

found in the Ashti and Pandharpur tanks almost exclusively, those of *A. culicifacies* being almost or entirely absent.

Adults have commonly been found during the winter and spring months in sheds, outhouses and quarters at Pandharpur, and also in the railway rest-house.

It cannot be said whether or not this species plays any rôle in the transmission of malaria on the line.

A few male specimens having the stem and lower branch of the fifth wing vein mainly pale were identified at the Central Malaria Bureau as specimens of *A. annularis*.

(6) *A. moghulensis*.

A. moghulensis has been found breeding throughout the course of the line in pools in the nullahs, along with *A. culicifacies* and *A. theobaldi*. It appears to be essentially a wild species, the larvæ thriving best in pools in connection with streams containing clean water, far from human habitations. They have been found in particularly large numbers in the ravines in the vicinity of Ramling, being as a rule most numerous at the end of the monsoon and during the winter months.

(7) *A. turkhudi*.

A. turkhudi has been found breeding, usually in scanty numbers only, throughout the course of the line. It occurs in pools in the nullahs, along with *A. culicifacies*, *A. stephensi* and sometimes *A. moghulensis*. It appears to be most numerous on the section Pandharpur to Miraj. The larval collections did not show any definite seasonal prevalence, but the greatest numbers were collected during the cold months. A single larva was found in the Pandharpur tank, and one adult female was captured in the railway colony there.

(8) *A. fluviatilis* (*A. listoni*).

A. fluviatilis is probably present throughout the course of the line, although it has not been found at every station. It has only been found in scanty numbers, however, except at certain places, as for instance in the small streams between Pangri and Kuslamb, where a large number of larvæ were observed on two occasions, though only a few were bred out.

No definite seasonal prevalence has been observed. In a disused, grass-edged well examined in May during the height of the hot season, it was the only species found breeding, but the most active breeding appears to take place at the end of the monsoon. It is the only species of which the larvæ have been found in streams where the current is swift. Clean water and grass along the margins seem to provide favourable conditions for its breeding. On the whole it is not a common species, and it is not considered to play an important rôle in the transmission of malaria on the line.

The following variations were observed in specimens identified as *A. fluviatilis* by the Central Malaria Bureau :—

(i) Specimens having the third vein entirely dark.

(ii) Specimens having the inner quarter of the costa with a pale interruption.

(9) *A. pallidus*.

A. pallidus, with the exception of a single specimen captured at Latur and a few from Dhulgaon, has only been found at Pandharpur and the neighbouring stations. It breeds freely along with *A. annularis* in the Pandharpur and Ashti tanks from the end of the monsoon, when there is a decrease in the breeding of *A. culicifacies*, until April. *A. pallidus* also breeds freely at the end of the monsoon in borrow-pits containing turbid water and generally decaying vegetable matter, along the course of the line at Pandharpur and the neighbouring stations. It also occurs in seepage pools and other small water collections.

No adults have been captured in living quarters; of the two specimens taken one was found in a shed and the other in a latrine.

(10) *A. hyrcanus* var. *nigerrimus*.

A. hyrcanus var. *nigerrimus* is probably present throughout the course of the line, but in its breeding habits it seems to be more fastidious than any of the species hitherto mentioned. There has always been a high mortality amongst the larvæ collected, and only a few have arrived at the adult stage. Its favourite breeding places are wells and shady pools, especially when the water surface is covered by decayed leaves. During the first years of my observations, it was also found breeding several times in a part of the Pandharpur tank where small trees and bushes afforded shade, but since the lowering of the water level in this tank the larvæ of this species have no longer been observed in it.

As far as the writer's observations go, this species has a definite seasonal breeding time, larvæ having only been collected during the last three months of the year.

(11) *A. barbirostris*.

A. barbirostris appears to be a more definite well breeder than *A. hyrcanus*. Otherwise, the above remarks regarding distribution and breeding conditions in respect of the latter species apply equally well to *A. barbirostris*. It has never been found breeding in Pandharpur tank. A single larva of this species was collected from a water-trough used by cattle at Kurduvadi, where the water was not very clean.

A male specimen with pale areas on the palpi was identified at the Central Malaria Bureau as being of this species.

(12) *A. maculatus*.

A. maculatus has a wide distribution in the area, having been encountered at Latur and the neighbouring stations, near Kurduvadi, and as far south-west as Athni Road. The larvæ were found in pools in the nullahs containing clear water, and in wells. This species has not shown any definite seasonal prevalence.

(13) *A. tessellatus*.

A. tessellatus is decidedly uncommon in this area. A blood-engorged female was caught in August in railway quarters at Kurduvadi, in a room

where four of the occupants were infected with benign tertian malaria. No larvæ of this species have been found in the neighbourhood. Two adults were bred out from larvæ collected in temporary pools near Pandharpur tank, and one from a swamp near Bohali station.

The outer half of the proboscis in the females captured was found not to be all white, as has been described.

(14) *A. jamesi*.

Three larvæ of this species were collected from pools in nullahs.

(15) *A. splendidus* (*A. maculipalpis* var. *indiensis*).

Two specimens of this mosquito were bred out from larvæ collected from pools in nullahs, in the month of February.

(16) *A. philippinensis*.

Two specimens of *A. philippinensis* were bred out from larvæ collected in pools near the river Sina, north-east of Mahisgaon. They were identified at the Central Malaria Bureau.

(17) *A. karwari*.

One female of this species was captured in a bath room in the Medical Officer's bungalow at Kurduvadi in the month of November. As the palpi were damaged it could not with certainty be distinguished from *A. majidi*; but seeing that the latter species has not been found in any neighbouring district, it was probably *A. karwari*. This specimen was sent to the Central Malaria Bureau for identification.

(18) *A. vagus*.

One larva of *A. vagus* collected from a nullah pool at Bohali in November was identified at the Central Malaria Bureau as being of this species*.

* *Addendum*.—Further collections were made at a few stations from September 1934 to January 1935 and the additional data have been incorporated in Tables II and III, and are summarised below :—

(1) *A. culicifacies*.—Adults were collected in railway quarters at Jath Road in November, and in those at Kurduvadi in December and January.

(2) *A. subpictus*.—Larvæ were collected from the Padmi tank at Pandharpur, and adults in railway quarters at Jath Road, in November.

(3) *A. stephensi*.—Collections of larvæ were made from the Padmi tank at Pandharpur in November and December. Adults were caught in railway quarters at Pandharpur in October, November and December, at Jath Road in November, and at Kurduvadi in December and January.

(7) *A. turkhudi*.—One adult each was caught in the railway quarters at Pandharpur and at Yedsi, and some more in those at Kurduvadi, in December. This species had not been found at the last station previously.

(10) *A. hyrcanus* var. *nigerrimus*.—Adults were collected from Kurduvadi railway quarters in December for the first time.

(18) *A. vagus*.—Larvæ were found in pools on the margins of the Padmi tank at Pandharpur in November. Previously this species had been recorded only from Bohali, the next station southwards.

Note.—Numbers before the species are the same as given in the text.

III. INCIDENCE AND DISTRIBUTION OF MALARIA.

1. GENERAL INCIDENCE.

The present study relates to the periods from April 1930 to March 1932, and from October 1932 to March 1934, inclusive. During those 3½ years, there were a total of 883 attacks of fever diagnosed as malaria among 699 individual patients:—384 benign tertian, 361 subtertian, 7 quartan, 11 mixed benign tertian and subtertian, 1 mixed subtertian and quartan and 119 diagnosed on clinical grounds.

Counting as above the total cases of malaria during the said period, the following percentages are obtained:—

Benign tertian	43 49
Subtertian	40 88
Quartan	0 79
Benign tertian and subtertian	1 25
Subtertian and quartan	0 11
Clinical malaria	13 48

If, however, the clinical and mixed cases be excluded, the following approximate percentages are found. These show the relative proportion of the three species of parasites on the Barsi Light Railway during the above periods:—

Benign tertian	51 per cent
Subtertian	48 per cent
Quartan	1 per cent

The 699 individual patients may be classified as follows:—

- (a) By age—416 men, 118 women, 165 children up to 12 years of age;
 (b) by race—Hindus 538, Muslims 95, Indian Christians 41, Anglo-Indians 18, Portuguese Indians 6, Europeans 1.

Five hundred and seventy persons had 1 attack each; ninety had 2 attacks; twenty-eight had 3 attacks; eight had 4 attacks; two had 5 attacks, and one, a little girl, was treated for 7 attacks during the period under review. As the actual sequence of attacks in each individual cannot be shown conveniently in a table, only the total figures are given in Table IV.

The observations may be summarised as follows:—

(a) Of the 90 persons who had two attacks, in 27 the attacks were both benign tertian, in 22 they were both subtertian and in 7 they were both 'clinical' malaria. In 18 one attack was benign tertian and one subtertian; in 10 one was subtertian and one 'clinical'; in 6 one was benign tertian and one 'clinical'.

(b) Of the 28 individuals who had three attacks, in 9 these were all benign tertian and in 4 subtertian. In 5 cases two attacks were benign tertian and one subtertian, while in 3 two were subtertian and one benign tertian. Two attacks of benign tertian and one of 'clinical' malaria were reported in 3 cases; one attack each of benign tertian, subtertian and 'clinical' malaria in 1 case; one attack of subtertian and two of 'clinical' malaria in 1 case; two attacks of subtertian and one of 'clinical' malaria in another. In one individual the first and second attacks were benign tertian and subtertian respectively, while in the third attack both species of parasite were found.

(c) Of the 8 individuals who had four attacks, 1 showed benign tertian parasites in all attacks and 1 subtertian. In two patients benign tertian attacks occurred three times and subtertian once. In 1 patient attacks of benign tertian and of 'clinical' malaria each occurred twice, while 2 patients had one attack of benign tertian and three of 'clinical' malaria, and 1 had three attacks of benign tertian and one 'clinical'.

TABLE IV.
Incidence of malarial attacks.

Number of attacks per person.	Number of persons attacked.	Total number of attacks.	NATURE OF ATTACKS.*					
			B. T.	S. T.	Qt.	B. T. + S. T.	S. T. + Qt.	Clinical.
1	570	570	226	254	7	9	1	73
2	90	180	78	72	30
3	28	84	48	28	..	1	..	7
4	8	32	17	6	9
5	2	10	9	1
7	1	7	6	1

* B. T. means benign tertian malaria, S. T. subtertian and Qt. quartan.

(d) Of the 2 patients in whom five attacks were recorded, 1 showed benign tertian parasites in the first four and both benign tertian and subtertian in the last, while in the other patient benign tertian parasites only were found in all the attacks.

(e) In the patient who was treated for seven attacks, the first six were benign tertian and the last subtertian.

TABLE V.

Years.	1930-1931.		1931-1932.		1932-1933. 6 months only.		1933-1934.	
	Modlimb-Latur.	Miraj-Ashti.	Modlimb-Latur.	Miraj-Ashti.	Modlimb-Latur.	Miraj-Ashti.	Modlimb-Latur.	Miraj-Ashti.
Total number of attacks in railway employees.	52	No figures available.	46	65	43	15	57	50
Number of individual employees treated for malaria.	46		41	59	41	15	52	47
Total number of days of unfitness caused by malaria.	278		258	431	207	105	245½	344
Average number of days of unfitness per attack.	5.3		5.6	6.6	4.8	7	4.3	6.9
Total number of employees	1,753		1,741		1,741		1,741	
Percentage of employees attacked by malaria.	..		5.7		..		5.7	
Average number of sick days lost per 1,000 of employees.	..		396		..		339	

For the purposes of medical aid the Barsi Light Railway line is divided into two sections. Patients at all stations from Miraj to Ashti are treated at the railway dispensary at Pandharpur, which is in charge of a Sub-Assistant Surgeon, whereas the patients from Modlimb to Latur attend at the railway dispensary at Kurduvadi, the headquarters of the Railway Medical Officer. As the railway is an economic undertaking, the number of days lost due to malaria is of considerable importance. The figures for the two sections of the line are shown separately in Table V.

2. YEARLY AND SEASONAL INCIDENCE.

The numbers of cases of malaria treated on both the sections of the railway line during the 3½ years' period under review are given in Table VI.

In order to obtain a uniform base for comparison the half-year period October 1932 to March 1933 has been excluded, Graphs I, II and III showing the monthly incidence during 3 full years.

TABLE VI.
Yearly incidence.

	1930-1931.		1931-1932.		1932-1933. Oct. to Mar.		1933-1934.	
	Modlimb-Latur.	Miraj-Ashti.	Modlimb-Latur.	Miraj-Ashti.	Modlimb-Latur.	Miraj-Ashti.	Modlimb-Latur.	Miraj-Ashti.
Benign tertian	39	120	39	77	18	10	50	31
Subtertian	42	84	33	52	27	24	47	52
Quartan	1	1	3	1	..	1
Benign tertian + subtertian	3	1	2	1	1	..	2	1
Subtertian + quartan	1
Clinical	3	22	1	20	23	1	12	37
Total	88	227	76	151	72	36	111	122
Total on the whole line ..	315		227		108		233	

An attempt has been made by careful study of the histories and by comparative clinical and blood examinations to indicate in each case whether it was likely to be a fresh infection or a relapse. The latter term is here used, for the sake of convenience, to cover 'recrudescences', 'relapses' and 'recurrences'.

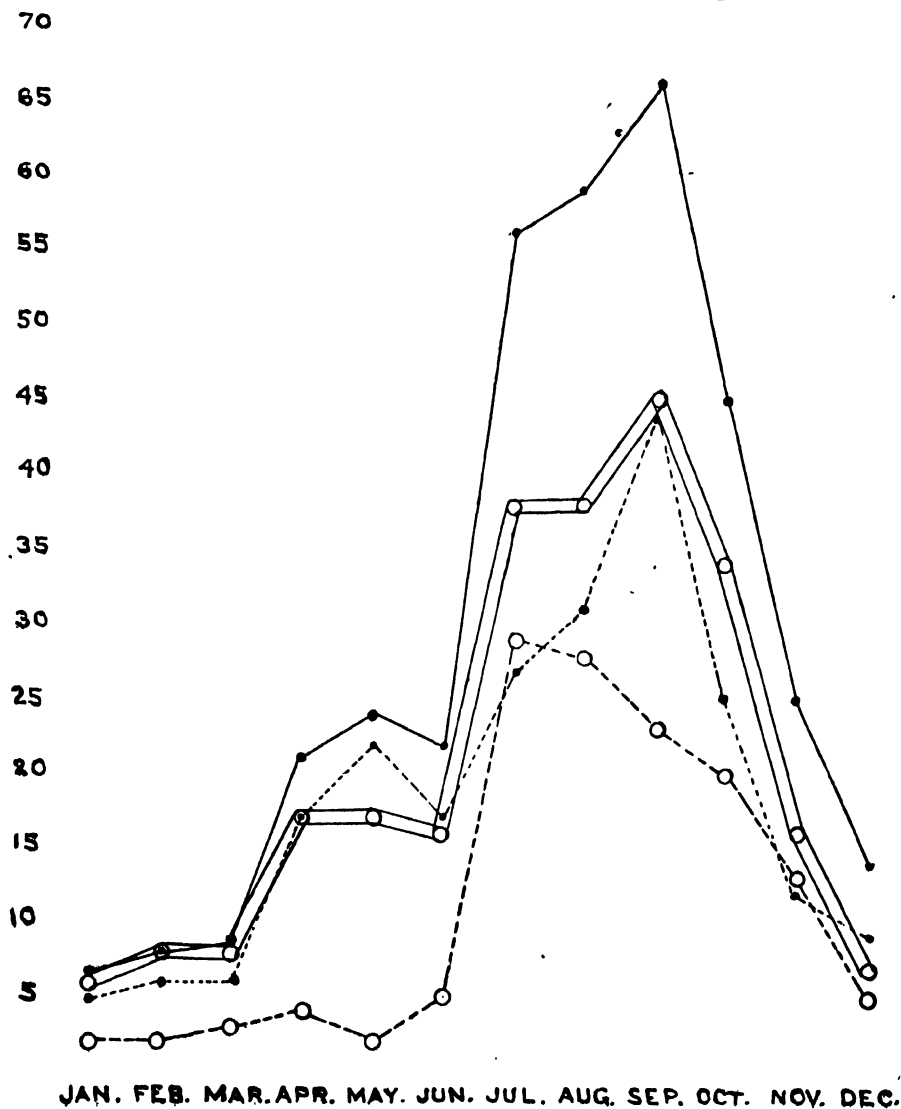
The unreliability of these figures is of course fully realised, but the knowledge possessed of the epidemiological conditions prevailing at each station, and the facts that the great majority of the cases were seen at the onset of the attacks and that their previous histories were usually well known, made this attempt possible with a certain degree of accuracy.

GRAPH I**BENIGN TERTIAN MALARIA MONTHLY INCIDENCE**

TOTAL BENIGN TERTIAN CASES ●—●

PRIMARY INFECTIONS ○---○ RELAPSES ●---●

GAMETOCYTES PRESENT ○—○



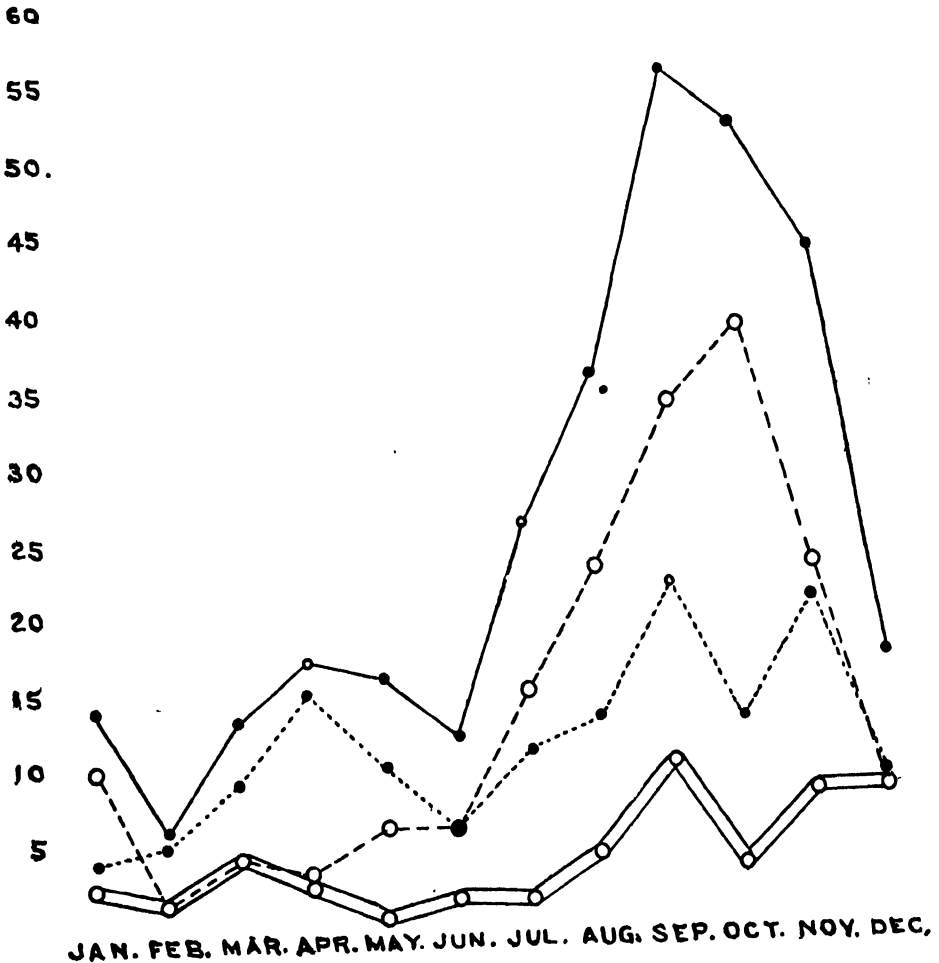
GRAPH II

SUBTERTIAN MALARIA, MONTHLY INCIDENCE

TOTAL SUBTERTIAN CASES —●—

PRIMARY INFECTIONS O--O RELAPSES●

GAMETOCYTES PRESENT ◻◻

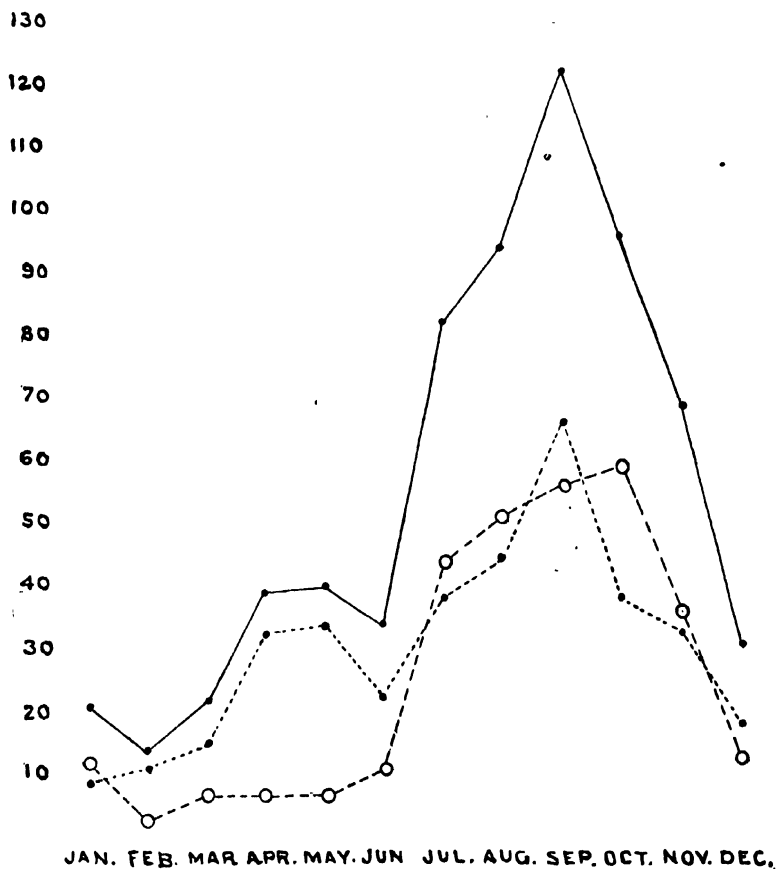


The cases were considered to be relapses where gametocytes were found on the first, second or third day of the fever, in patients having suffered from previous attacks, and where there was no reason to suspect a prolonged incubation period with the possible development of gametocytes during the latency.

GRAPH III

BENIGN TERTIAN & SUBTERTIAN MALARIA MONTHLY INCIDENCE

TOTAL CASES —●— PRIMARY INFECTIONS ○---○
RELAPSES ●-----●



Cases diagnosed clinically, as well as mixed infections, have been excluded from the graphs.

In Graph I is shown the monthly incidence of the benign tertian attacks during the 3 years taken together. The total low incidence during December, January, February and March is strikingly brought out, as well as the comparatively high relapse rate during April and May, the hottest months of the

year, with a second peak in September. The highest total incidence was during the rainy months July, August and September.

A study of Graph II, in which the monthly attacks due to subtertian malaria during the same period are plotted, shows a different state of affairs. Subtertian malaria is really active only during 5 months of the year, from July to November; there is no real relapse season during the hot weather; fresh infections and relapses follow a similar course. Both the varieties show a maximum total incidence in September, though the largest number of primary attacks was observed in October in the case of subtertian malaria.

On Graph III the benign tertian and subtertian cases are plotted together. In this connection a reference should be made to Table I, where monthly rainfall figures are given.

3. REMARKS ON THE DISTRIBUTION OF MALARIA IN DIFFERENT STATIONS AND ON ITS EPIDEMIOLOGY.

It has already been mentioned that the previous history of each patient was usually known. This was possible through the keeping of permanent records for each individual. The grouping together of the records of all the members of each family allowed data regarding familial infections to be available at a glance. On these records every transfer, absence on leave, etc., are also noted, so that the dates of residence and the length of stay at each place are in most instances accurately known. Moreover, treatment for any complaints is nearly always given in one of the railway dispensaries and invariably recorded, and the epidemiological conditions at each station are, to some extent, known. It was found possible, therefore, to note down in a number of cases the origin of infection with a fair degree of probability. In a certain number of the patients, however, and nearly always in the case of running staff on duty, such as drivers, firemen and guards, this was not possible.

The cases where a probable origin of infection could be given are recorded in Table VII for the 3½ years' period under review. Attacks which appeared to be recrudescences, relapses or recurrences are not shown in this table.

In 66 cases the infection was evidently contracted at places outside the area of railway administration. The great majority of these were contracted in the Deccan, at or near neighbouring stations of the main G. I. P. Railway line, or in villages in Hyderabad State; 7 were contracted near Poona by employees belonging to the Auxiliary Force of India and attending training camp there.

Regarding the percentage figures of the incidence at the various stations, the following remarks must be made:—(a) The population figures are those of the census taken in 1931 and are of course only approximate; and (b) though the number of employees does not vary appreciably, the number of relatives and guests residing with them often fluctuates greatly. Thus, during a spleen survey made by the writer in July 1934, 741 occupants of railway quarters were found at Pandharpur, whereas the 1931 census gives only the figure of 598.

A certain number of persons, shown as having had attacks of malaria at the stations, do not reside there or only do so occasionally. This applies especially to Kurduvadi, where a large number of employees live in Kurduvadi

TABLE VII.

Malaria incidence on the railway.

Stations.	NATURE OF ATTACKS.*					Total cases.	Railway population.	Number of attacks per hundred of railway population (approximate percentage).
	B. T.	S. T.	B. T. plus S. T.	Qt.	Clinical.			
Miraj ..	1	2	3	230	1.3
Bolwad ..	2	3	5	26	19
Arag ..	3	3	39	7.7
Athni Road ..	1	4	1	6	33	18
Sulgaon ..	1	2	1	4	27	15
Dhulgaon	2	1	3	36	8.3
Kavathe Mahankal	1	..	1	33	3
Langarpeth ..	5	6	11	21	52
Dhulgaon ..	1	2	3	31	10
Gulvanchi	9	..
Jath Road ..	8	4	1	..	1	14	70	20
Javla	1	1	29	3.4
Wasud ..	5	7	12	34	35
Sangola ..	2	1	1	4	51	7.8
Bamani ..	4	5	1	10	39	26
Bohali ..	2	7	9	27	33
Pandharpur ..	132	136	4	..	32	304	598	51
Babhulgaon ..	8	3	11	38	30
Ashti ..	9	10	19	49	39
Modlimb	6	..	1	1	8	36	22
Padsali	4	4	30	13
Laul ..	5	6	2	(13)†	(9)	22
Kurduvadi ..	30	31	13	74	1,443	5.1
Chink Hill ..	3	2	1	6	149	4
Mahisgaon	2	2	39	5.3
Shendri ..	7	8	1	16	50	32
Uplai ..	1	1	25	4
Barsi Town ..	4	2	3	9	258	3.5
Kuslamb ..	1	3	4	20	14
Pangri ..	7	9	..	1	..	17	48	35
Ramling ..	9	2	11	30	36
Yedsi	3	1	..	2	6	37	16
Kalamb Road	6	..	1	1	8	73	11
Dhoki	41	..
Thair	1	1	7	14
Palsap	3	..	1	..	4	63	6.3
Murud ..	5	7	1	13	38	34
Neoli ..	3	6	9	42	21
Owsa Road ..	2	9	11	47	23
Hangul ..	1	2	3	25	12
Latur	2	2	270	0.7

* B. T. means benign tertian infection, S. T. means subtertian and Qt. means quartan.

† Of these 13 cases only 2 resided on the station, the remaining 11 lived in Laul village, and 5 worked at Kurduvadi.

village and the surrounding villages. Of the 13 cases shown for Laul station, only 2 resided there; the 11 remaining lived in Laul village and 5 of them were working at Kurduvadi.

The percentage incidence shown for Kurduvadi is much too high, the majority of the cases really originating in the neighbouring villages. Out of the 74 attacks, there were hardly 25 in which the infection was likely to have been contracted in the Kurduvadi railway colony, and this gives a percentage of 1.7, instead of 5.1. It will be seen, therefore, that the percentage is increased by the number of railway employees and their dependents, resident at villages in the vicinity of Kurduvadi and coming for treatment, as a considerable number of these patients are not included in the population figure of 1,443, given for the railway colony. It has not been found possible, however, to calculate the percentage in any other way.

At Pandharpur, the situation is quite different. Practically all the cases among the residents of Pandharpur, treated at the railway dispensary, have contracted their malaria in the railway colony. During the period under review, there were hardly more than 4 or 5 amongst the cases tabulated in which the infection might have been brought from the town or from a neighbouring village.

The railway population itself, as a human reservoir, cannot, of course, be entirely dismissed. Up to 1929 it had been nearly everywhere a factor of importance, and is still so at one station. However the following operations during the last 6 years have evidently reduced indigenous malaria to a minimum at every station except Pandharpur :—

- (a) blood examination of every case of fever;
- (b) quinine and plasmoquine treatment in what must be considered as adequate dosage, with 'follow-up' treatments in nearly every case;
- (c) the introduction of a new rule on the railway by which every man who is given work, whatever his status, must be sent for medical examination prior to his employment, and
- (d) frequent examinations of all the occupants of railway quarters.

The writer is unfortunately not in possession of any literature with reference to the flight range of *A. culicifacies* in the Deccan. This species is practically the only carrier on this railway needing consideration. Breeding places within 5 furlongs (about 1 km.) of railway quarters have been considered as being likely to be easily within the flight range of the vectors concerned. This distance has been taken somewhat arbitrarily for the purpose of classification, while it is admitted that the range of dispersion may be far greater.

In studying the factors governing the incidence of malaria at the railway stations, these can be classified into 4 groups, taking only into consideration the presence of anopheline carriers and outside human reservoirs.

(A) *Stations located near outside human reservoirs, with anopheline carriers breeding in the neighbourhood.*

Over half the stations (23) belong to this category*. The name of the nearest potential reservoir is given within parenthesis, with its distance from the station. From a number of observations made in several of these villages they are in fact probably all actual reservoirs.

- (i) Bolwad (Bolwad village, 5 furlongs); (ii) Athni Road (Belankhi, 5 furlongs); (iii) Sulgare (Salgar village, 3 furlongs); (iv) Dhulgaon (Dhulgaon

* For details of the actual breeding places, the reader is referred to the unpublished portions of Part II 'Anopheline Mosquitoes'.

village, 5 furlongs); (v) Langarpeth (Dhalevadi, 1 furlong); (vi) Dhalgaon (Dhalgaon village, 1 furlong); (vii) Jath Road (Valekhind, 2 furlongs); (viii) Sangola (station at outskirts of village); (ix) Bohali (Khardi, 4 furlongs); (x) Pandharpur; (xi) Babhulgaon (Ahir Babhulgaon, 5 furlongs); (xii) Ashti (Ashti village, 4 furlongs); (xiii) Modlimb (Morlimba village, 5½ furlongs); (xiv) Laul (Laul village, 1 furlong); (xv) Kurduvadi (Kurduvadi village, about 1 furlong from nearest railway quarters); (xvi) Mahisgaon (Mahisgaon village, 3½ furlongs); (xvii) Uplai (Khandvi, 5 furlongs); (xviii) Kuslamb (Kuslamb village, 5½ furlongs); (xix) Pangri (Pangri village, 4 furlongs); (xx) Yedsi (Yedsi village, 2 furlongs); (xxi) Kalamb Road (Tadvala, 2½ furlongs); (xxii) Thair (Buknawari, 1½ furlongs); and (xxiii) Palsap (Palsap village, 4 furlongs).

Assuming that female anophelines do make excursions during the period of their infectivity, the transmission of the malaria at these stations can obviously occur even in the absence of human carriers at the stations themselves. It need not be discussed further.

A special consideration must, however, be made of conditions at Pandharpur. Although the railway colony is really situated about 7 furlongs from any inhabited part of the town, this station must be included in the first category, because thousands of pilgrims spend evenings and nights at the station during the two biggest fairs of the year. At these times breeding of *A. culicifacies* in the Padmi tank is at its height, and thus fresh mosquito carriers are no doubt infected in large numbers. This maintains a constant supply of infection to the inhabitants of the railway colony.

(B) *Stations located close to outside human reservoirs, without carriers breeding in the neighbourhood, or with very scanty and occasional breeding only.*

Only 3 of the largest stations, where urban conditions prevail, belong to this category, namely (i) Miraj; (ii) Barsi Town and (iii) Latur. In these three places the stations are situated on the outskirts of the towns.

At Miraj and Latur, no carrier has been found breeding closer than 1 mile from the station. At Barsi Town very scanty numbers of the larvæ of *A. culicifacies* and of *A. stephensi* were found from two to three furlongs from railway quarters. These were apparently living there precariously amongst numerous *Culex* larvæ in polluted water.

Of these three stations, one is a terminus, one is a junction station with a line of a different gauge, and the third is a terminus for some of the local services. As the wagons are cleaned out at such stations, the possible introduction of anopheline carriers by the trains must also be considered. Repeated searches by the writer in the trains have, however, always been negative.

It may be seen from Table VII that the incidence of malaria is small at these stations.

(C) *Stations located far from outside human reservoirs, with anopheline carriers breeding in the neighbourhood of the stations.*

Eight stations may be classified under this heading:—(i) Gulvanichi (Dudebhavi, 1 mile 7 furlongs); (ii) Wasud (Akola, 7 furlongs); (iii) Bamani (Manjari, 7 furlongs); (iv) Padsali (Padsali village, 1 mile 3½ furlongs); (v) Ramling (Ramling temple, 7 furlongs); (vi) Dhoki (Dhoki village, 1 mile);

(vii) Murud (Taurajkhera, 1 mile 3 furlongs); and (viii) Owsa Road (Murud Akola, 1 mile 2 furlongs).

Quite fortuitous circumstances govern the incidence of malaria at these stations. Where no human carrier resides at the station, they may remain entirely free from any malaria year after year. This was the case with Gulvanchi and Dhoki during the period under review. If, on the other hand, a human carrier be introduced, either (a) as frequently happens when relatives arrive from villages in the district, ill themselves or bringing a child or two with enlarged spleens and a history of previous attacks, or (b) by the arrival of a new employee whom the railway authorities have failed to send for medical examination, there may then be a sudden outbreak. As many as 5 or 6 cases may even occur in the same family or block of quarters at a station, where not a single case has been known for several years. This was observed for instance at Ramling, where the infection unfortunately lingered on after the initial outbreak, a series of apparently primary attacks taking place subsequently, at different times and apparently from a single source of infection.

Familial outbreaks are particularly common at these stations.

(D) *Stations located far from outside human reservoirs and without carriers breeding in the neighbourhood or with very scanty and occasional breeding.*

Seven stations can be placed in this class:—

(i) Arag (Erandoli, 1 mile 6 furlongs); (ii) Kavathe Mahankal (Pimplachivadi, 1 mile); (iii) Javla (Hanumantgaon, 7 furlongs); (iv) Chink Hill (Akalgao, 1 mile 2 furlongs); (v) Shendri (Vangarvadi, 7 furlongs); (vi) Neoli (Borgao Buzurg, 1 mile 3 furlongs); and (vii) Hangul (Harangul Buzurg, 1 mile 4 furlongs).

Anopheline mosquitoes have been searched for at these stations but not found. As their presence can, however, not be entirely excluded, the occasional transmission of malaria is probably possible, but in nearly all the cases there has been good evidence to show that the disease was imported from one of the surrounding villages.

As seen in Table VII the incidence is low at 4 of these stations.

Regarding Shendri with its incidence of 32 per cent, there are a number of employees and dependents who frequently spend evenings and nights at Vangarvadi village, which is highly malarious. When ill, such persons remain at the station for the sake of obtaining treatment. The same remark holds good for Neoli and Hangul, the infection being usually imported into these stations by gangmen residing permanently or temporarily in malarious villages in the surrounding area.

4. DISTRIBUTION OF INFECTIONS IN RELATION TO SPECIES OF PARASITES.

The data given in Table VII furnish an approximate idea of the incidence of infections due to the 3 species of malarial parasites. It is, however, almost certain that both benign tertian and subtertian infections occur in all the villages surrounding the 41 stations, and probably in approximately equal proportions.

Quartan malaria, on the other hand, is decidedly uncommon. Eight cases of this infection were observed during the period under review. To give a more complete idea of the distribution of quartan malaria, to these may be added 4 other quartan attacks treated at a later period. The probable origin of these 12 cases was as follows:—

(i) Kavathe Mahankal (Ranjni village); (ii) Khadaki near Mangalveda, about 15 miles east of Bamani station; (iii) Pandharpur; (iv) Modlimb (Bavi village); (v) Ujjani, 4 miles south-west of Laul, and about 7 miles south-west of Kurduvadi; (vi) Uplaval, about 10 miles north-west of Kurduvadi, and 3 miles south of Kem, a station on the main line between Kurduvadi and Dhond; (vii) Mahisgaon (Papnas village); (viii and ix) Pangri (Gholevadi and Pangri villages); (x) Kalamb Road (Vagholi village); (xi) Palsap (Palsap village); and (xii) Pandare on the Nira Canal, 8 miles west of Baramati and 20 miles south of Dhond (a mixed infection).

5. SPLEEN SURVEYS.

The results of the spleen surveys at the 5 largest stations are given, and those of 2 smaller stations are also given for the sake of comparison (Table VIII). With the exception of the surveys at Miraj and Barsi Town, they were all made by the writer. All the persons were examined in the recumbent position.

It was found advisable to include infants and toddlers, because of their susceptibility to malaria, and the comparatively unimportant number of other pathological conditions accompanied by splenic enlargement occurring in small children in the Deccan. The upper limit for children was taken at 12 years, and when the age was unknown, those showing a definitely adolescent habitus were counted as adults.

TABLE VIII.

Spleen rates.

CHILDREN:				ADULTS.	
Stations.	Date.	Number examined.	Spleen rate (per cent).	Number examined.	Spleen rate (per cent).
Miraj ..	July 1934	55	5.5	76	0
Pandharpur ..	May 1931	146	37	244	23
" ..	July 1931	274	11	427	10.5
Kurduvadi*	February 1931	117	11
" ..	February 1932	121	9
" ..	February 1933	165	14
Barsi Town ..	August 1933	55	0	142	3.5
Kalamb Road ..	November 1934	27	0	24	4
Palsap ..	November 1934	36	2.8	33	3
Latur ..	October 1934	120	4	152	3.3

* School children only.

In comparing the findings at Pandharpur in 1931 and 1934, it must be noted that the first survey was made during the dry and hot weather, when spleen rates are considered to be at their lowest. On the other hand, the second survey was made during the monsoon, when fresh infections were taking place, and after a period of preventive plasmoquine treatment on a large scale had intervened (*vide* Lindberg, 1932*a*, 1932*b*).

As regards the higher spleen rate in the school children at Kurduvadi in 1933, a certain number of ignorant parents had previously refused to send their children for examination, but were persuaded to do so in that year. The result was that several children with enlarged spleens were then seen for the first time.

IV. CLINICAL OBSERVATIONS ON MALARIA IN THE DECCAN.

The reason why this study has been made is the ever-growing conviction amongst malariologists that malaria is a disease showing important variations in different regions. These are shown both in its clinical behaviour and its reactions when therapeutically influenced, whether these be questions of different strains of parasites or due to constitutional factors in the human hosts. The apparent total absence of any clinical or parasitological reports from the whole of the area under investigation seems fully to justify the labour involved. The only locality near any of the stations of the B. L. Railway from where any data seem to be available is Sholapur, as referred to by Knowles and Senior White (1930). These data are very different from those obtained by the writer in the same district.

The present notes do not in any way aim at giving a complete account of malaria in the Deccan. They only deal with certain of its aspects. It is hoped, however, that their publication will induce workers in this and other parts of the world to collect data on similar lines, as this can so easily be done with a minimum of staff and equipment.

1. OBSERVATIONS ON THE BLOOD.

(a) PRESENCE OF GAMETOCYTES.

Table IX shows the number of cases in which gametocytes were found per total attacks and per individual patients, during the period of 3½ years under review. The mixed cases are included and shown under separate headings for each species of parasite. These figures would tend to show that children constituted a more important reservoir of infection than adults, as is usually considered to be the case. This is markedly more so in the case of the subtertian parasite than in that of the benign tertian.

TABLE IX.

Nature of attack.	Total attacks.	NUMBER OF INDIVIDUAL PATIENTS.			TOTAL ATTACKS SHOWING GAMETOCYTES.		PRESENCE OF GAMETOCYTES IN INDIVIDUAL PATIENTS.			
		Adults.	Children.	Total.	No.	Per cent.	Adults.		Children.	
							No.	Per cent.	No.	Per cent.
Benign tertian ..	395	239	81	320	277	70	165	69	61	75
Subtertian ..	373	251	84	335	51	13·7	31	12	20	24
Quartan ..	8	6	2	8	8	100	6	100	2	100

No patient in which crescents once were found showed crescents again at the time of a subsequent attack. All the cases of quartan malaria were relapsing infections or ones of long standing.

In 7 cases of febrile attacks, gametocytes were found alone in the absence of any asexual forms; 3 were benign tertian, 3 subtertian and 1 quartan.

(b) SEASONAL PREVALENCE OF GAMETOCYTES.

The findings during 3 full years (October 1932 to March 1933 being excluded) are shown in Table X and have also been plotted on Graphs I and II.

TABLE X.
Seasonal prevalence of gametocytes.

	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.
<i>Plasmodium vivax</i> . Number of cases	7	8	9	21	24	22	56	59	66	45	25	14
Gametocytes present in—	6	8	8	17	17	16	38	38	45	34	16	7
Percentage (approximate).	86	100	90	81	71	73	68	64	68	75	64	50
<i>Plasmodium falciparum</i> . Number of cases	14	6	13	18	16	12	26	36	56	52	44	17
Gametocytes present in—	2	1	4	2	..	1	1	4	10	3	8	8
Percentage (approximate).	14	17	30	11	0	8	4	11	18	6	18	47

Table X shows the greatest prevalence of benign tertian gametocytes during the months when primary attacks were least numerous. On the other hand their presence in subtertian cases showed a very irregular seasonal distribution, which would hardly allow of any conclusions being drawn.

(c) RELATIONSHIP BETWEEN THE PRESENCE OF MATURE SCHIZONTS OF THE BENIGN TERTIAN PARASITE AND THE HEIGHT OF THE TEMPERATURE OF THE PATIENT.

It is said in certain textbooks that mature schizonts are present in the peripheral blood only during the febrile stage. The series of cases reviewed here do not fully bear out this statement.

Mature schizonts of *P. vivax* were found in 40 attacks in 38 individual patients (about 10 per cent) and those of *P. malariae* in 4 attacks (50 per cent).

Their relationship to the temperature recorded when the blood films were taken is shown in Table XI.

TABLE XI.

		Temperatures up to 99.5°F. (Class a).	Temperatures from 99.6°F. to 101.5°F. (Class b).	Temperatures of 101.6°F. and over. (Class c).
Cases .. {	Adults	2	8	14
	Children	7	3	6
Percentages {	Adults	22	73	70
	Children	78	27	30
Percentages of Total ..		22.5	27.5	50

Of the 20 cases in Class (c), 8 showed temperatures from 101.6°F. to 102.9°F. and 12 had temperatures from 103°F. to 105°F.

The above figures would show that mature schizonts are often found when temperatures are high, as there were 12 cases out of the 40 in which temperatures ranged from 103°F. to 105°F. On the other hand, the presence of mature schizonts is not necessarily accompanied by fever as nearly a quarter of the cases were afebrile, and this was more often the case in children than in adults.

(d) TOTAL LEUCOCYTE COUNTS.

Forty-five counts were made, of which 39 were from men, 1 from a woman and 5 from children. The total count for these cases averaged 6,400 leucocytes per c.mm. In 25 benign tertian infections the average was 7,200; in 19 subtertian it was 5,800 and in 1 quartan the count was 6,800.

The relationship of the total counts to the height of the pyrexia is shown in Table XII.

TABLE XII.

	TEMPERATURES (IN °F.).			
	Up to 99.5.	From 99.6 to 101.5.	From 101.6 to 102.9.	Of 103 and over
Number of counts ..	20	11	5	9
Average total white cells per c.mm.	6,300	6,600	8,300	6,600

The lowest count was 2,300 in a man aged 30 with a subtertian infection and a temperature of 98°F. The highest count was 13,000, in a man aged 30 with a benign tertian infection and a temperature of 101.2°F.

The lowest temperature observed was 97°F. in 3 cases, in which the counts were respectively 4,800, 8,700 and 9,000. The highest temperature was 104.8°F. in a 5-year-old child with benign tertian malaria, and this case showed a total leucocyte count of 10,600.

In 195 cases, apparent decreases, increases, or normal numbers of the leucocytes were recorded as estimated from examination of the thin and thick films, an estimation which with experience can be made with a fair amount of accuracy. In 67, or about 34 per cent, the number appeared to be about normal; in 94, or about 48 per cent, they had evidently decreased, and in 34, or about 18 per cent, there was apparently an increase.

(e) DIFFERENTIAL LEUCOCYTE COUNTS.

The results of average counts according to Schilling's classification are given in Table XIII for benign tertian and subtertian cases. These are shown separately for adults and for children up to 12 years, without regard to the occurrence of temperatures or other concomitant conditions, such as respiratory catarrh, gastro-intestinal disturbances, intestinal parasites, etc. However, cases, in which a definite associated disease liable to influence the differential count was present, have been excluded. Two hundred cells were counted in each case and all the counts made by the writer, who has been familiar with Schilling's method for the last 8 years. Averages were first calculated separately for men and for women, but the differences were found to be very slight. Men and women are, therefore, here shown together (Table XIII). The youngest child included in the series was 3 years old.

TABLE XIII.

Differential leucocyte counts recorded irrespective of the occurrence of temperature conditions, etc.

CASES.	BENIGN TERTIAN MALARIA.						SUBTERTIAN MALARIA.					
	ADULTS—129.			CHILDREN—46.			ADULTS—134.			CHILDREN—35.		
	Highest counts.	Average counts.	Lowest counts.	Highest counts.	Average counts.	Lowest counts.	Highest counts.	Average counts.	Lowest counts.	Highest counts.	Average counts.	Lowest counts.
Types of leucocytes.												
Neutrophiles ..	91	58.39	18	75	45.26	19	90	55	20	79	47.17	17
Myelocytes	0.07	0.08	..	5	0.09	..	2	0.08	..
Juveniles	3.07	1.65	..	26	3.38	..	22	2.85	..
'Stab' forms	33.88	25.21	..	70	33.28	3	55	28.92	8
Segmented forms	21.37	18.32	..	57	18.25	2	50	15.32	1
Lymphocytes ..	73	34.05	6	70	46.74	16	72	38	9	83	45.27	17
Large mononuclears ..	17	5.50	..	27	6.07	1	16	5.03	..	16	5.50	..
Eosinophiles ..	19	1.84	..	12	1.67	..	30	1.87	..	26	1.90	..
Basophiles ..	2	0.22	..	2	0.26	..	2	0.10	..	1	0.16	..

As seen from Table XIII, the total average counts both in adults and children do not show any appreciable divergence in the two species of parasite. They have therefore been studied together in their behaviour in relation to the temperature (Table XIV).

TABLE XIV.
Differential leucocyte counts (benign tertian, subtertian and mixed injections)
in their relationships to temperatures.

NUMBER OF CASES.	TEMPERATURES UP TO 99.5°F.									TEMPERATURES FROM 99.6°F. TO 101.5°F.									TEMPERATURES OF 101.6°F. AND OVER.								
	ADULTS—122.			CHILDREN—37.			ADULTS—67.			CHILDREN—18.			ADULTS—80.			CHILDREN—28.											
Types of leucocytes.	Highest counts.	Average counts.	Lowest counts.	Highest counts.	Average counts.	Lowest counts.	Highest counts.	Average counts.	Lowest counts.	Highest counts.	Average counts.	Lowest counts.	Highest counts.	Average counts.	Lowest counts.	Highest counts.	Average counts.	Lowest counts.									
Neutrophiles	79	46.98	20	75	41.57	17	91	60.31	27	74	45.27	23	90	67.68	18	79	53.57	20									
Myelocytes	2	0.07	nil		0.10	nil	5	0.10	nil	nil	..	nil	5	0.07	nil	2	0.10	nil									
Juveniles ..	20	2.21	nil	8	1.89	nil	18	3.14	nil	10	1.83	nil	19	4.51	nil	22	3.46	nil									
'Stab' forms	56	26.60	5	55	24.83	6	69	36.06	8	51	24.56	11	70	41.97	7	52	32.44	19									
Segmented forms.	51	18.10	1	40	14.75	1	66	21.01	3	59	18.88	5	64	21.13	2	40	17.57	1									
Lymphocytes	72	43.77	10	83	50.19	20	64	33.08	8	70	47.25	21	73	27.87	6	64	39.00	16									
Large mono-nuclears.	16	6.69	nil	27	5.54	nil	17	5.33	nil	13	6.05	1	12	3.75	nil	16	5.26	nil									
Eosinophiles	19	2.36	nil	10	2.43	nil	30	1.14	nil	4	1.27	nil	6	0.62	nil	26	1.89	nil									
Basophiles	2	0.20	nil	1	0.27	nil	2	0.14	nil	1	0.16	nil	2	0.08	nil	2	0.28	nil									

The averages in Table XIV show that a rise of temperature is accompanied by an increase in the neutrophils and a concomitant decrease in the lymphocytes; the higher the temperature the more marked were the variations. This takes place both in adults and children, but, apparently owing to the normally higher rate of lymphocytes in children, it is much less marked in them. Besides this general increase of the neutrophils, there is a shift of the index to the left, which is also much more marked in adults than in children.

The large mononuclears are practically uninfluenced by the temperature, though the averages for adults show a gradual decrease with the rise of the temperature. This is not, however, well brought out by the records of the children in this series.

Eosinophiles follow the usual behaviour of these cells in febrile diseases, i.e., they decrease with the rise of temperature.

These findings are partly at variance with statements in certain textbooks dealing with European standards, but in order to judge of their real significance, the normal differential leucocyte count in healthy Deccan Indians should of course be known. A study of this question has been undertaken by the writer and will be published elsewhere.

Examinations of the stools*, to determine the incidence of helminthic and protozoal infections, were carried out in 243 out of the 269 cases in this series. This was done to discover whether such conditions had any bearing upon the average number of eosinophile leucocytes found. As a result of these examinations, such conditions were considered to be unimportant; eosinophiles were here found more numerous in the absence of febrile attacks, and they usually decreased in patients suffering from malaria.

(f) HÆMOGLOBIN ESTIMATIONS.

By means of the Tallqvist scale, hæmoglobin readings were made in 236 cases:—110 benign tertian, 120 subtertian, 3 quartan and 3 mixed infections (Table XV).

TABLE XV.

Records of percentages of hæmoglobin.

Cases.	PERCENTAGES OF HÆMOGLOBIN.													
	100		90		80		70		60		50		40	
	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
Benign tertian	13	12	53	48	27	24.5	9	8.2	7	6.4	1	0.9
Subtertian ..	7	6	48	40	37	31	15	12.5	8	6.4	3	2.5	2	1.6
Total examined	21	9	104	44	64	27	25	10.6	16	7	4	1.6	2	0.8

*The details of these examinations are given in the original, but have been deleted as irrelevant to the point under discussion.—(Editor).

A comparison between the relative percentage of hæmoglobin in benign tertian and in subtertian malaria infections is given in Table XVI.

TABLE XVI.

Relative percentages of hæmoglobin in infections with benign tertian and with subtertian malaria.

Cases.	Percentages of hæmoglobin.						
	100	90	80	70	60	50	40
Benign tertian (per cent) ..	65	52.5	42	37.5	46.5	25	0
Subtertian (per cent) ..	35	47.5	58	62.5	53.5	75	100
Number of cases . .	20	101	64	24	15	4	2

From Tables XV and XVI it is evident that a marked anæmia is more often found in subtertian malaria than in benign tertian infections.

2. SPLENIC ENLARGEMENT.

Out of the total of 883 attacks of malaria, the condition of spleen was noted in 878 (the unrecorded cases being 3 benign tertian and 2 subtertian attacks). The details of the approximate percentages are given in Table XVII.

TABLE XVII.

Condition of the spleen.

	Benign tertian.	Percentage.	Subtertian.	Percentage.	Quartan.	Percentage.	Benign tertian + subtertian.	Percentage.	Clinical malaria.	Percentage.	+ Subtertian quartan.
Spleen not enlarged.	182	48	172	48	2	28.5	7	64	76	64	..
Spleen palpable	125	33	107	30	3	43	1	9	33	28	..
Spleen 1 finger-breadth below costal margin.	24	6.2	30	8	1	9	4	3	1
Spleen 2 finger-breadths below costal margin.	33	8.6	34	9.5	2	28.5	2	18	4	3	..
Spleen 3 finger-breadths below costal margin.	12	3	9	2.50	2	2	..
Spleen 4 finger-breadths below costal margin.	3	0.7	7	2
Spleen reaching to umbilicus.	2	0.5

Both in the attacks due to benign tertian and to subtertian infection, splenic enlargement was detected in 52 per cent of cases. A spleen rate of 71.5 per cent was found in quartan infections, while in mixed infections (due to benign tertian and subtertian malaria) and in 'clinical' cases the rates were 36 per cent in each instance.

The above figures are interesting in view of the statement usually made that the spleen is more often enlarged in benign tertian than in subtertian attacks, percentages such as 60 to 70 in benign tertian and 30 in subtertian having been reported from other parts of the world.

Percentage figures of the condition of the spleen in 570 individual patients, each having suffered from one attack, were calculated, but found to be almost identical with those of the total attacks and the details are therefore omitted. Of the 90 persons who had had two attacks each, the size of the spleen was stationary in 50 and showed variations in the two attacks in 40. Of the 28 persons who had suffered from three attacks, the splenic enlargement was stationary in 7 and varied in the remaining 21.

As no conclusions can be drawn from the variations in the size of the spleen during the various attacks, the details are omitted. In persons with several attacks there was, however, some evidence to show that an increase in the size of the spleen from one attack to another pointed to a fresh infection.

3. HEPATIC INVOLVEMENT.

This series covers 740 cases (benign tertian 381, subtertian 359).

Only rough clinical observations on the presence of enlargement of the liver, icterus, bile pigments in the urine and ascites were recorded. Any functional liver tests and determinations of the bilirubin in the plasma were not done in any of the cases, nor is the question of the significance of increased urobilinogen entered into here. The findings are shown in Tables XVIII and XVIII(A).

" TABLE XVIII.
Hepatic involvement.

	Enlarged liver.	Hepatic or cholecystic congestion.	Choluria.	Icterus.	Ascites.
Benign tertian .. (381 attacks).	5 (children).	6 (5 adults, 1 child).	5	3 (2 adults, 1 child).	..
Subtertian .. (359 attacks).	12 (2 adults, 10 children).	11 (9 adults, 2 children).	1	1 (adult).	1 (child) (liver also enlarged).
Benign tertian + subtertian (11 attacks).	1 (adult).

TABLE XVIII(A).
Degree of hepatic enlargement in relation to the type of infection.

	Benign tertian.	Subtertian.	Benign tertian + subtertian.
Liver palpable (nipple line) ..	1	4	1
Liver 1 finger below costal margin (nipple line).	1	4	..
Liver 2 fingers below costal margin ..	2	3	..
Liver 3 fingers below costal margin ..	1	1	..

The percentage figures of evident hepatic involvement in this series are 3.67 in the benign tertian attacks and 6.68 in the subtertian attacks.

Under the heading 'hepatic or cholecystic congestion' are noted those cases in which definite pain and muscular resistance were found in the region of the gall-bladder during the attacks in persons known not to have shown this sign previously, and this disappeared under the influence of quinine medication. Nearly all these cases suffered from gastro-intestinal disturbance and 11 of them (5 benign tertian and 6 subtertian) also had transient albuminuria with granular casts and renal epithelial cells. The positive Gmelin tests (excluded from the total percentage calculations) were obtained in 5 of these patients and the sixth in one of the icterus cases.

Of the attacks in which hepatic or cholecystic congestion was noted, enlarged spleens were found in 9 out of the 11 subtertian cases, and in 5 out of the 6 benign tertian attacks.

The spleen was enlarged in all but one of the cases showing definite enlargement of the liver.

Bile pigments in the urine were not present in any of the 17 cases in which there was definite enlargement of the liver.

Urobilinogen in the urine was increased in all the benign tertian cases where the liver was enlarged, and also in 10 of the 12 subtertian cases.

An interesting feature is the much greater frequency of enlargement of the liver in children than in adults (15 children out of the 18 cases), and in subtertian than in benign tertian attacks. The enlargement of the liver disappeared or decreased in all the cases which were kept under treatment and observation. Six were cases of malarial cachexia with very low hæmoglobin values. Crescents were present in the blood in 5 out of the 12 subtertian cases.

Attacks of malarial hepatitis with ascites are sufficiently uncommon to warrant some short notes on such a case in this series:—

A male child, aged 6 years, was brought to the dispensary on 25th December, 1930, on account of 'swelling of the abdomen'. The main findings were: Blood—subtertian rings and crescents; hæmoglobin, 50 per cent. Spleen—3 fingers sub-costal enlargement; Liver—1 finger sub-costal enlargement. Marked abdominal distension with free peritoneal fluid. Collateral venous circulation in lower thoracic and upper abdominal regions. Urine—urobilinogen, positive; Gmelin's test, negative; albumin, absent; sugar, absent. Generalised adenitis. *Ascaris* ova in stools. There was no fever.

Quinine hydrochloride 0.25 gm. thrice daily with plasmoquine 0.005 gm. twice daily were given for 4 days, and 8 *Ascarides* were removed. On 29th December, the spleen was felt 2 finger-breadths below the costal margin. Ascites was the same. Quinine was continued alone in the same dosage for 8 days more.

On 1st January, 1931, the spleen was no longer palpable, nor could the liver be felt in the nipple line. The peritoneal fluid did not seem to have decreased.

Quinine hydrochloride 0.30 gm. was then given twice daily and an iron arsenic mixture for 5 days. On 11th January, 1931, the ascites had evidently decreased and the collateral circulation was much less evident. Quinine, iron and arsenic were continued up to 28th January. On 24th January, there was only a small amount of free fluid in the peritoneal cavity and no distended cutaneous veins.

The child was examined again on 27th February during a routine examination of school children. The notes made at the time were:—Slight abdominal distension, no evidence of fluid. Liver and spleen negative.

Regarding the cases with icterus, this condition was slight in two patients and unaccompanied by choluria. In the third, a man aged 54, it was very

marked. It appeared suddenly and was accompanied by a large amount of albumin with numerous granular casts; Gmelin's test was strongly positive and there was a very marked increase of urobilinogen. Vomiting was severe. The jaundice as well as the albuminuria disappeared entirely, when a total of 2 gm. of quinine had been ingested. The last case of icterus was in a man aged 30 also suffering from secondary syphilis. Gmelin's test was negative and urobilinogen increased. The jaundice disappeared under quinine and sulfarsenol medication.

4. GLYCOSURIA.

Malarial attacks occurred in two patients well known to have diabetes. In 3 other patients a transient glycosuria was observed during the attacks (2 benign tertian, 1 subtertian). In one of the benign tertian cases, a watchman aged 50, both Gerhardt's and Rothera's tests were positive. Severe albuminuria with casts was also present, which, as well as the sugar, disappeared entirely after four days of atabrin and plasmoquine treatment.

5. UROBILINOGEN.

Its frequency was investigated in the fresh urines of 292 individual patients (203 men, 31 women and 58 children).

The findings are summarised in Table XIX.

TABLE XIX.

Incidence of increased urobilinogen in the urine in malarial attacks.

Type of case.	Total cases examined.	CASES SHOWING UROBILINOGEN.		Type of infection.	Total cases examined.	CASES SHOWING UROBILINOGEN.	
		Number.	Per cent.			Number.	Per cent.
Men ..	203	185	91	Benign tertian. Subtertian	144	132	91
Women ..	31	27	87		148	129	87
Children..	58	49	84				

Faintly positive tests were obtained in approximately 20 per cent; markedly positive in approximately 70 per cent; and very strongly positive in approximately 10 per cent of the positive cases.

Tests were only made once a day, within 3 hours of the morning meal in about half the cases, and on an empty stomach in the other half. When the urine was found positive, it usually remained so throughout the febrile attack, whereas a negative reaction was sometimes found only once and was later followed by positive tests.

It may be seen from the above figures that negative reactions are somewhat more often met with in subtertian than in benign tertian attacks. Although the number of tests done in children is small, it would appear that negative reactions are found more frequently in children than in adults. This is also what may be expected taking into consideration the physiological urobilinuria which Bang

(1929) showed to be less marked in children than in adults, without, however, giving any explanation.

An analysis of the negative urobilinogen cases failed to reveal any evident connection between this condition and factors such as duration of the fever, height of temperature, evidence of gross liver pathology, amount of blood destruction (as evidenced by the percentage of hæmoglobin), gastro-intestinal disturbances and carbohydrate intake*.

Diarrhœa was, however, present in 3 cases, and it is usually considered that urobilinogen will decrease or disappear from the urine when there is increased intestinal motility. On the other hand, 3 patients complained of constipation, in which condition urobilinogen is more often found to be increased. Two patients were seen on the first day of the fever, and thirteen on the second, before the increased urobilinogen became evident. On the contrary urobilinogen is more often found to have increased already on the first and second days of the fever. Eleven of the patients who showed no urobilinogenuria had evidence of catarrh of the respiratory tract, in which condition increased urobilinogen is a common finding in the absence of malaria.

No case showed icterus or presence of bile pigments in the urine. Enlargement of the liver and probable congestion of the gall-bladder were, however, present in 2 cases, but there was no evidence of obstruction of the common bile duct.

The urine of one of the cases, in which urobilinogen was absent on the third day of the fever, was again tested on the fifth day and then found positive.

In a case in which urobilinogen was absent on the fifth day, it had been found increased on the third day of the fever during a subtertian attack one month previously when a temperature of 99·6°F. had been recorded. Two years later the same patient was seen on the first day of a malaria attack with a temperature of 103·4°F. and increased urobilinogen. He complained of nausea and vomiting during all the three attacks.

In order to interpret the cases in which there was no increase of urobilinogen, an explanation should, in the first place, be furnished of the evident increase found in the large majority of malaria cases. A satisfactory explanation of this phenomenon has apparently not yet been given though it is generally considered to be due to an insufficiency of the parenchymatous liver cells, whether absolute and directly brought about by the circulating parasites or their metabolic products, or relative and consecutive to the increased destruction of red blood cells by the plasmodia. In view of our ignorance regarding the origin of urobilin, it is by no means certain that the increased urinary excretion of urobilinogen is the expression of hepatic dysfunction. Some authors have shown that malarial attacks are accompanied by hypoglycæmia. Therefore, the fact of the increased urobilinogen observed in cases of carbohydrate restriction and the physiological increase during fasts (Bang, 1929; Forsgren, 1931) seem to indicate a simple relationship to a depletion in glycogen of the hepatic cells during malarial attacks.

*The details of the observations made on these cases are tabulated in the original manuscript.—(Editor).

6. ALBUMINURIA.

Albumin was tested for (by Purdy's heat test) in every attack, but careful notes on its presence or absence, and on the constituents of the sediment, were only kept in a series of 143 (101 men, 12 women, 30 children) (Table XX).

TABLE XX.
Albuminuria.

Infection.	Number of cases.	Albumin absent.	ALBUMIN PRESENT.		Granular casts.	Renal epithelial cells.	Red blood cells.
			Traces only.	Distinct amounts.			
Benign tertian	67	42 (63 per cent).	11	14	25	25	2
Subtertian ..	76	41 (54 per cent).	13	22	35	35	5

In all these cases albumin was known to have been absent from the urine prior to the febrile attack, and no case has been included in which the albuminuria was associated with the presence of pus. There was no attack in which the albumin was found to persist after the subsidence of the febrile symptoms.

Granular casts and renal epithelial cells were present in all the cases where a trace or a definite amount of albumin was found. A fair number of granular casts and renal epithelial cells were also found in urines not showing any albumin. These have not, however, been recorded in the table, as the urinary sediments were not examined in every one of the albumin-negative cases. The casts were nearly always of the coarsely granular variety. Epithelial tube casts and blood casts were also found in several cases. Small polygonal markedly granular renal epithelial cells, each with a large nucleus, were invariably present. In most cases, also, were found the large clear round cells, often showing fatty degeneration, which are considered to originate from the calices of the kidney.

Piriform and small round cells with central nuclei were also present in many of the cases.

About 46 per cent of the subtertian attacks were accompanied by albuminuria in this series, and only about 37 per cent of attacks of benign tertian malaria.

7. HÆMOGLOBINURIA.

Only one case of hæmoglobinuria was recorded.

This occurred in a gangman about 50 years old, who reported sick on 22nd September, 1933, complaining of fever with chills, which had started the previous day. His temperature was 100·8°F. The blood films showed numerous benign tertian trophozoites, mature schizonts and gametocytes. The urine contained albumin with casts and renal epithelial cells, but no red blood corpuscles. The temperature became subnormal the following

day. On 24th September, after the administration of a total of 1·8 gm. quinine hydrochloride, the patient started passing turbid reddish-brown urine. On the next day the urine was a deep crimson colour and gave a strongly positive benzidine reaction, but contained no red blood cells. The amount of quinine actually absorbed was, however, doubtful, as Mayer's test was only faintly positive on 23rd and 24th September. The patient's treatment was changed to plasmoquine and the urine became free from hæmoglobin on 28th September. He was able to resume his duties on 8th October.

8. SYMPTOMATOLOGY*.

(a) CHIEF COMPLAINT.

The chief complaint for which medical aid was sought by the patients was particularly noted in an unselected series of 307 cases. Small children were excluded from this series, which was mostly adults. There were 159 cases of benign tertian and 148 of subtertian malaria.

An endeavour was made to obtain in the patient's own words a single symptom as being the predominant complaint. However, a large number gave a combination of two symptoms as the chief reason for seeking medical aid.

The symptom '*fever*', either alone or in combination with other symptoms, was the chief complaint in 84 per cent of the cases. In this series '*fever*' was the predominant complaint slightly more often in benign tertian than in subtertian attacks. The symptom-complex, '*cold fever*', was the commonest, and was complained of in 208 cases, but it must be mentioned that the majority of patients attended for acute attacks of the disease.

'*Shivering*' was an outstanding symptom only in conjunction with '*fever*', and was reported in 96·2 per cent of such cases. In the absence of noticeable fever it was mentioned as a chief complaint in only 3·8 per cent of the cases.

'*Pain*' or '*aching*' was complained of in about 12 per cent of cases, headache being the commonest form. Pain as a predominant symptom occurred in about the same frequency in both benign tertian and subtertian malaria.

Gastro-intestinal symptoms, either alone or in conjunction with fever, chills or cough, were reported in 9·4 per cent of cases. Such complaints were commoner with subtertian infections, and vomiting was the chief one.

Only 2·3 per cent of the patients complained of *respiratory* symptoms, such as cough or coryza, in combination with some other complaint.

Giddiness and *general malaise* were each complained of by two patients.

Urticaria was the chief complaint of two patients, both having spring relapses of benign tertian malaria. *Yellowishness of the eyes* was the sole complaint of one boy in whom fever was never observed, although the blood showed *P. vivax* and the spleen was enlarged.

(b) THE OCCURRENCE OF FEVER AND THE RANGE OF TEMPERATURE.

Temperature charts were kept of the majority of cases treated at Kurduvadi, but, as they all received treatment, a study of the fever curves is of no interest. When there was no complication by other ailments the fever was nearly always absent on the third day after treatment started.

* The original manuscript deals with this subject in very great detail, and it has been found necessary to abridge this for publication.—(Editor).

Observations were made on a series of 372 benign tertian attacks occurring in 292 individuals, of 353 subtertian attacks in 296 persons, and of 8 quartan cases (7 pure infections and one mixed with subtertian).

Among the benign tertian patients, fever was present in all but 2 attacks. Among the subtertian cases fever was absent in 8 cases only, and in 2 among the quartan patients. All the latter 10 patients in whom fever was not found gave histories of pyrexia at a more or less remote period.

TABLE XXI.
Range of temperature.

Infection.	Number of attacks.	TEMPERATURE BELOW 97°F.		TEMPERATURES BETWEEN 97°F. AND 104°F.		TEMPERATURES OF 104°F. AND OVER.	
		Number of cases.	Per cent about	Number of cases.	Per cent about	Number of cases.	Per cent about
Benign tertian	372	1	0.2	327	88	44	11.8
Subtertian ..	353	15	4.4	297	84	41	11.6

The temperatures were taken orally except in the case of the smaller children, where the rectal method was used. The very large majority of patients showed temperatures between 99°F. and 102°F. when first examined. The extremes of temperature observed have been noted. The highest temperature recorded was 106°F. in both the benign tertian and the subtertian series. The lowest temperature in the latter series was 93°F. in a case of malarial cachexia, and in the former was 96.6°F.

(c) ANALYSIS OF OTHER SYMPTOMS.

This series comprises 381 benign tertian attacks (men 228; women 50; children 103), and 357 subtertian attacks (men 217; women 49; children 91). The data for young children were, of course, somewhat unreliable and incomplete.

Aching.—Pain was the commonest symptom complained of by the patients:—headache, most common; ‘burning of the eyes’, fairly common; ‘aching of the whole body’, such as muscular, bone and joint pains. In subtertian cases aching was a feature of the prodromal stage, or of the hours following the defervescence of fever, or both.

Shivering was the next symptom in order of frequency, usually with a more or less pronounced rigor. In a few cases it was entirely absent (35 benign tertian and 38 subtertian infections in adults).

Perspiration, apart from the stage of defervescence of fever, was not a common complaint.

Fainting was observed in 4 men and 1 child.

Convulsions were seen on 3 occasions, twice being in a child with two attacks of benign tertian malaria at 3 weeks’ interval.

Herpes was only observed in 6 men of the benign tertian series, and was labial or naso-labial. The comparative rarity of herpes in this series is in contrast to the statement of Nocht and Mayer (1918) that benign tertian infections are constantly accompanied by herpes, nor was it found in any of the cases of malarial cachexia (all subtertian infections) in which it is said by Manson-Bahr (1923) to be very common. On the other hand, herpes was only encountered in one of the subtertian attacks, thus corroborating the statement of Nocht and Mayer (1918) that it is much less common in such infections. This, moreover, was a genital herpes and its connection with the malaria attack was uncertain.

Abdominal pain, apart from pain on palpation, was usually complained of as epigastric distress or a more or less well-defined pain around the umbilicus. One subtertian attack had abdominal pain simulating appendicitis.

Diarrhoea was more common in subtertian than in benign tertian attacks.

Dysenteric symptoms.—In patients with no evidence of bacillary or amoebic infections, the passage of blood and mucus was apparently related to acute febrile attacks. These signs disappeared rapidly under anti-malarial treatment, with or without preliminary purgation.

Flatulent dyspepsia was the main symptom in four subtertian cases with cachexia. The stools showed signs of intestinal carbohydrate indigestion.

Catarrh of the upper respiratory tract was recorded in about 20 per cent of subtertian cases, as compared with 17 per cent of benign tertian. This frequency of catarrhal respiratory signs in malarial attacks should be borne in mind in relation to the differential diagnosis from influenza. The writer has not found the occurrence of increased urobilinogen excretion in the urine of much assistance in such cases, as he finds this condition also occurs in the majority of febrile colds accompanied by pharyngitis and tracheo-bronchitis. This is in agreement with the findings of Grönberg and Grönberg (1931).

Cardiac signs.—Persistent tachycardia, extra-cardiac murmurs and bradycardia were observed in different cases.

Dysuria.—Retention of urine was present as a prominent symptom in two male children about 7 years of age, suffering from subtertian malaria. It was relieved by anti-malarial treatment.

Impaired hearing was complained of by two patients who had not taken either quinine or salicylates. No local condition could be found to explain it. It was observed in both instances during afebrile periods.

Urticaria occurred in two patients.

9. MORTALITY.

Three deaths, apparently due to malaria, were observed—(a) In a subtertian infection in a man, aged 55 years, there was a severe intestinal hæmorrhage; (b) a man, aged about 60 years, with a mixed subtertian and benign tertian infection, was admitted in an extremely exhausted state and expired after 4 hours; and (c) a 2-year-old child with benign tertian malaria developed coma.

V. RECOMMENDATIONS.

The question of how to reduce the incidence of malaria along the Barsi Light Railway must be dealt with under two headings :—(1) What the railway can do; and (2) what should be done outside railway limits.

1. ANTI-MALARIAL MEASURES WITHIN RAILWAY LIMITS.

The line was constructed originally and stations laid out without expert advice being taken on the malaria problem. Any proposal for shifting Class I stations* from their present sites is hardly a practical one. It is most unlikely to be carried out and will therefore not be further considered here. At such sites nearly all breeding places of importance are outside railway limits, and, this being so, there is practically no hope of the railway being in a position to carry out the required engineering or sanitary undertakings. This rules out any anti-larval operations by the Railway Company at stations of Classes I and III*. On this account at stations of Class I (Pandharpur excepted), measures have to be confined to those of such limited scope as early detection and adequate treatment of every individual case of malaria, destruction of adult mosquitoes, inducing the better paid employees to use mosquito nets, and supervision of the floating population (relatives and guests).

At Pandharpur with its uncontrollable and unlimited supply of human carriers which is renewed every season with the comparative simplicity of its mosquito breeding problem, the malaria campaign must in the first place take the form of preventing the anopheline carrier from breeding. The writer's recommendation, to empty the Padmi tank and connect the Takli nullah with the Bhima river by means of a concrete channel, is probably the cheapest and most efficient way of dealing with the situation, as far as the railway colony is concerned. It must, however, be borne in mind that, as far as the surrounding area is concerned, this is likely to be a half-measure, if not done in conjunction with other operations. Making conditions in a certain area unsuitable for the breeding of a species such as *A. culicifacies*, is likely to result in this species finding other breeding places. Such will be found in seepage water below embankments in the catchment area of the Takli nullah, and in wells and pools at and around Takli village. Under such conditions the great endemicity of malaria in the Pandharpur railway colony might possibly be transferred to Takli village, where its ravages would be far greater than in the railway colony, owing to the lack of medical aid in this village. To those who take a cynical view of the question, this may not matter very much, as sickness and deaths from malaria in Takli village will probably attract as little attention from outside sources as they do in thousands of other malarious villages in India. From a medical and humanitarian point of view, however, this would of course mean that the scheme was a failure.

The stocking of the Padmi tank with larvivorous fish cannot be of any practical value, as the bulk of the breeding takes place in very shallow water at the margins of the tank and in disconnected temporary pools, in which situations no fish can reach the larvæ.

At stations of Classes II and IV, the measures of limited scope already mentioned are of real value and sufficient to keep the incidence of malaria at

* Vide classification of stations on p. 73.

a minimum level. As to the control of actual cases, we, of course, have to depend under such conditions in a large measure on the patients reporting to the railway medical department for treatment when ill. This is unfortunately not always the case, when wives and other dependents of employees are concerned at the 3 stations of Class II, where there are numerous private practitioners. This fact is a considerable handicap in the railway's anti-malarial campaign. None of these practitioners use a microscope, and they usually treat patients, believed to be suffering from malaria, by the oral giving of quinine in 2-grain doses after the subsidence of the fever, or by a single or a few intramuscular quinine injections. The common result is that, when such patients eventually report to the railway doctors for treatment, they have been ailing for weeks and months and are usually carriers of benign tertian gametocytes, or of crescents.

As regards stations of Class III, the obvious measure here is the use of prophylactic treatment by plasmoquine and atabrin, or plasmoquine alone, before the onset of the season of active mosquito breeding. The difficulty lies in persuading persons who are not ill, and who seldom understand the meaning of preventive medication, to take the doses, and also in finding staff who will conscientiously supervise the actual ingestion of each dose.

Anti-gametocyte treatment is no doubt also of value at stations of Classes II and IV. At Pandharpur a reduction in the number of fresh infections and in the spleen rate was evidently achieved, though, of course, there is at Pandharpur no hope of eradicating the malaria by this measure alone for the reasons already set forth.

A special difficulty on this railway are the frequent and numerous transfers of staff from one station to another. In this way a number of gametocyte carriers, mostly originating from Pandharpur, are every year disseminated all along the line. A great desideratum is, therefore, to find time and staff to administer a course of prophylactic treatment to every person on transfer from Pandharpur to another station.

The practice of employing gangmen as 'temporary' workers, often for long periods, without the knowledge of the medical department, adds considerably to the uncertainty of malaria control on the railway. Such gangmen, or members of their families, are frequently suffering from periodical attacks of malaria.

2. ANTI-MALARIAL MEASURES OUTSIDE RAILWAY LIMITS.

The suggestions which follow will perhaps be considered by some workers as entirely impracticable for financial reasons. It is fully realised that the question of eradicating malaria from rural India, or at any rate from the Bombay Presidency and Hyderabad State, is not likely to be taken in hand or even seriously considered, say, within the next 50 years to give a modest figure. It is believed, however, that every student of the causes of the poverty and general apathy in Indian villages will admit that the frequent attacks of fever, the chronic ill-health and the debility caused by malaria are some of the main factors at the root of the evil. If India is to take the place she should occupy amongst the civilised nations of the world, and the Indian country-side is to cease to be a death-trap to its inhabitants and to casual visitors, the malaria problem, amongst others, of rural India must be tackled one day.

The advantage gained by the opening of a dispensary in every village, where a Sub-Assistant Surgeon, either without a microscope or with one but without the inclination to use it, would dole out a few doses of quinine to his fever cases, would no doubt be very small compared to its cost.

With the present conditions of great poverty, the writer is of opinion that anti-larval measures should be considered in the first place.

The flight range of *A. culicifacies*, *stephensi* and *fluviatilis* in the Deccan must be investigated, although probably only that of *A. culicifacies* need be considered as being important.

To any one who has observed throughout the different seasons of the year the breeding grounds of *A. culicifacies* near the villages, it stands to reason that oiling, chemical larvicides or biological methods cannot here be efficiently and generally used. As practically only the pools in the nullahs are of importance, the solution seems to be that the beds of such nullahs should be converted into concrete channels for a distance of a mile or so (depending on the flight range of *A. culicifacies*) in each direction of every village. The ultimate hope would be that the *A. culicifacies* females will develop zoophilism when human blood is no longer within easy reach.

Once the breeding has been reduced around the villages, the benefits of a dispensary, where expert anti-malarial treatment can be obtained, will be considerably increased. But the all-important question at the bottom of the whole problem of the prevention of malaria is that of education, and this brings us down to the question of what the children should be taught in the primary schools. It is hoped that the day will come when every child will learn the elementary facts of hygiene and sanitation, and how the common diseases of his country are spread, even if this knowledge should be gained at the expense of some other literary attainment.

Here Government has full powers, and the question of increased expenditure hardly arises.

VI. SUMMARY.

(1) An account is given of the breeding places, as well as the geographical and seasonal distribution, of eighteen species of *Anopheles* found along the Barsi Light Railway.

(2) The incidence and distribution of malaria on the railway during a period of 3½ years has been studied in its various aspects. As a result of this investigation, a classification of the railway stations on epidemiological grounds is given and the results of spleen surveys recorded.

(3) The incidence of gametocytes in adults and children and the seasonal prevalence of such parasites are tabulated.

Other parasitological and hæmatological findings are tabulated and discussed, such as the relationship of the presence of mature schizonts to the height of the temperature, average total and differential leucocyte counts and their relationship to the height of the fever, and hæmoglobin values in benign tertian and subtertian cases in adults and children.

(4) Percentages of splenic enlargement in relation to the type of infection are given. These are not in agreement with those reported from other parts of the world.

(5) Cases with hepatic involvement are analysed. The question of the urobilinogenuria in malaria is discussed and an explanation of its increase offered.

(6) Percentage figures of the incidence of albumin in the urine are given in a series of benign tertian and subtertian malaria cases.

(7) Clinical findings are reported and partly analysed, with the idea of giving an account of the symptomatology of malaria, as met with on the Deccan plateau.

(8) Recommendations for the reduction of the prevalence of malaria are made.

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ANOPHELES BREEDING IN RELATION TO RICE CULTIVATION IN LOWER BENGAL.

BY

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INTRODUCTION.*

THERE has been a tendency during recent years among certain malariologists to associate the paddy fields* in the proximity of human habitations with the malariousness of a place. That is to say, the rice fields have been considered by many as the chief source of Anopheline output in a locality, the water accumulating in these fields furnishing the necessary breeding surfaces for these mosquitoes. This suspicion has naturally induced certain municipalities and other corporate bodies responsible for public health to prohibit wet cultivation within a mile or a half of their jurisdiction.

Rice being the staple food of this province, this prohibition might cause great distress to the people, and the matter, therefore, deserves serious consideration from the public health authorities of the country. The mere presence of rice swamps might not be sufficient to indicate that the place would be malarious. As we can understand, this would greatly depend on the extent to which the cultivation is found to afford breeding facilities to those Anophelines which are known to transmit malaria.

But, unfortunately, we are not fully aware what exact rôle is being played by the rice swamps under normal circumstances on the life and distribution of these carrier species of Anophelines. Wide divergence of opinion on this point exists amongst different workers, some claiming their complete dissociation in the causation of malaria while others consider the rice fields as the main source of trouble. Hardly any apology, therefore, is needed for reporting work on this problem.

* 'Paddy' is the Indian name for rice.

This investigation was taken up primarily at the suggestion of Dr. S. N. Sur, Assistant Director of Public Health, who has helped the author in various other ways while the work was in progress. My thanks are also due to Dr. R. B. Khambata, Director of Public Health, Bengal, for the constant encouragement I have received from him. To Lieut.-Colonel A. D. Stewart, I am especially grateful for his valuable criticisms.

METHODS.

The work was undertaken in October 1932 and was continued till the end of September 1934, thus the results enumerated here embody two years observations as to the state of actual breeding of Anophelines in the paddy fields. For comparative purposes, the incidence of Anopheline larvæ in the more or less permanent water collections in the villages of this particular area, was also carefully recorded. As the latter study was already in progress at the time of commencement of the paddy field observations, the figures for the villages will be shown from the beginning of 1932. Three villages, *viz.*, Sonarpur, Ukhila and Sitola, all within the jurisdiction of the police station of Sonarpur, and about 12 miles away from Calcutta, were selected for this purpose. This choice was made chiefly on account of the large number of paddy fields surrounding the villages, and because of their proximity to the Sonarpur Field Malaria Observatory of the Public Health Department, Bengal. The area may be considered as typical of lower deltaic Bengal.

Two sets of experiments were undertaken, in one, fortnightly examinations of every water collection in the three named villages were carried out, and in the other, similar observations were made on the water collections accumulating in the paddy fields only, in the outskirts of these villages during the paddy-growing season. The water collections in the villages consisted of a large number of tanks, ponds, ditches and drains of various dimensions. These collections were more or less overgrown with aquatic vegetation of all sorts. Those of the paddy fields, however, were comparatively free from floating vegetation, and the water was somewhat clear.

DESCRIPTION OF THE PADDY FIELDS.

Sur (1931) has given a detailed account of the various types of paddy fields and the methods of rice cultivation in vogue in the different parts of Bengal. I shall, therefore, refer to the type of cultivation of this area only in brief.

'Aman' or autumn paddy is mainly grown in this part of Bengal. The land is ploughed before the rains set in, and with the first shower the ground is prepared for sowing, which is usually done in June. When the seedlings appear in this prepared land, and after a heavy rainfall, the young plants are transplanted at definite distances in the water-logged soil about the month of July. The period of transplantation depends entirely on the time of commencement of the monsoon, as the heavy rains are of vital importance to the vigorous growth of the plants. The plots hold from two inches to above one foot of water during the paddy-growing season.

TABLE I.
Relative prevalence of *Anopheles* in the paddy fields.

Locality.	Total paddy fields considered.	Total larva bottles examined.		<i>A. hyrcanus</i> var.	<i>A. nigerrimus</i> .	<i>A. barbrosistris</i> .	<i>A. vagus</i> .	<i>A. subpictus</i> .	<i>A. varuna</i> .	<i>A. acutus</i>	<i>A. namayi</i> .	<i>A. annularis</i> .	<i>A. philippinensis</i> .	<i>A. tesellatus</i> .	<i>A. culicifacies</i> .	<i>A. pallidus</i> .	All species.
Sonarpur	52	1,146		56.7	11.7	9.0	2.8	6.2	5.2	0.3	7.5	0.4	0.1	:	:	:	100
			Average number of larvæ per 10 bottles.	26.78	4.85	2.34	0.4	3.14	2.78	0.14	2.67	0.27	0.04	:	:	:	43.41
Okhila ..	34	437		49.4	16.3	6.3	1.4	14.3	6.3	1.4	3.3	0.3	0.8	:	:	:	100
			Average number of larvæ per 10 bottles.	24.32	8.51	3.0	0.16	9.63	4.32	0.93	1.83	0.22	0.09	:	:	0.08	53.09
Sitola ..	50	651		45.6	24.3	6.7	2.3	11.2	1.6	0.3	7.9	100
			Average number of larvæ per 10 bottles.	20.18	7.67	3.56	0.37	4.66	0.95	0.08	3.36	40.84

TABLE II.
Relative prevalence of Anophelines in the village water collections.

Locality.	Number of water collections considered.	Total larvæ bottles examined.	<i>A. hyrcanus</i> var. <i>nigerrimus</i> .	<i>A. barbirostris</i> .	<i>A. vagus</i> .	<i>A. subpictus</i> .	<i>A. varuna</i> .	<i>A. aconitus</i> .	<i>A. ramsayi</i> .	<i>A. annularis</i> .	<i>A. philippinensis</i> .	<i>A. lessellatus</i> .	<i>A. culicifacies</i> .	<i>A. pallidus</i> .	All species.
Sonarpur	182	6,124	37.3	17.2	18.3	6.4	14.2	1.7	3.1	1.6	0.2	0.02	0.01	..	100
			Average number of larvæ per 10 bottles.			3.3	9.37	1.22	2.05	0.98	0.1	..	0.01	..	61.62
Ukhila ..	150	6,861	33.35	21.9	15.25	1.45	14.8	1.85	6.65	3.8	0.85	0.01	0.005	0.02	100
			Average number of larvæ per 10 bottles.			0.48	8.38	1.1	3.46	1.91	0.46	0.008	0.002	0.01	50.16
Sitola ..	65	2,940	19.10	15.36	40.12	11.1	9.48	0.6	0.3	3.9	..	0.05	0.08	..	100
			Average number of larvæ per 10 bottles.			4.32	3.84	0.31	0.15	1.59	..	0.02	0.03	..	40.50

DETAILS OF OBSERVATIONS.

The results detailed herein are based on the examination of 42,549 larvæ (37,672 from the villages and 4,877 from the paddy fields) from 533 breeding places, of which 136 were paddy fields (52 in Sonarpur, 34 in Ukhila and 50 in Sitola) and 397 village tanks, etc. (182 in Sonarpur, 150 in Ukhila and 65 in Sitola). The total collections represented at least twelve different species:—*A. hyrcanus* var. *nigerrimus* Giles, *A. barbirostris* Van der Wulp, *A. subpictus* Grassi, *A. vagus* Dönitz, *A. varuna* Iyengar, *A. aconitus* Dönitz, *A. ramsayi* Covell, *A. annularis* Van der Wulp, *A. philippinensis* Ludlow, *A. pallidus* Theo., *A. tessellatus* Theo., and *A. culicifacies* Giles. The species occurring in the area, together with their relative numerical frequency, are shown in Tables I and II. It will be seen that the last three species occurred only casually in the areas under investigation. It will also be noted from the tables referred to, that *A. culicifacies* was entirely absent from the paddy fields, and in the village water collections its number was insignificant. *A. pallidus* was far less common, and never prevailed, in Sitola. Both *A. pallidus* and *A. tessellatus* were recorded from the paddy fields on one or two occasions only.

The relative prevalence of the various Anopheline larvæ in each of the villages and paddy fields in the corresponding villages for the entire period of investigation, i.e., from January 1932 to the end of September 1934, has been shown in Chart I. It will be seen that *A. hyrcanus* is definitely the predominating species in the areas, excepting in the village Sitola where *A. vagus* predominated. Nearly 50 per cent of the total larval catch in the various paddy fields was represented by the former species. Excepting *A. culicifacies*, which never occurred in the paddy fields and only infrequently in the village collections, the carrier species noted to be breeding in the areas in order of intensity were *A. varuna*, *A. annularis* and *A. philippinensis*. In Sitola, however, the last-named species was absent, and in the other two areas it prevailed in scanty numbers only, being always below 1 per cent of the total catch.

In the paddy fields of Sonarpur, Ukhila and Sitola, *A. varuna* formed 6.2, 14.3 and 11.2 per cent respectively of the total collections. In the corresponding village collections the rates were found to be 14.2, 14.8 and 9.4 per cent respectively. Thus, excepting in Sitola, the breeding of *A. varuna* was less abundant in the paddy fields. The intensity of *A. annularis* breeding in the village water collections stood at a lower level than that of *A. varuna*, but the former species was noted to prevail in greater numbers in the paddy fields, except in Ukhila. The percentages of prevalence of *A. annularis* in these fields at the three places, Sonarpur, Ukhila and Sitola, were 7.5, 3.3 and 7.9, as opposed to 1.6, 3.8 and 3.9 respectively in the villages.

In Chart II, the proportionate distribution of the larvæ, per 10 standardised larvæ bottles brought for examination in the laboratory, has been shown. Each bottle contained the catches as collected in about ten dips. A consultation of the chart depicting the gross findings of the entire investigation will show that the larval rates in the paddy fields varied in the different villages, being 53 in Ukhila, 43 in Sonarpur and 41 in Sitola. The breeding was, however, more prolific in the Sonarpur village water collections, where the rate was found to be as high as about 62 per 10 bottles examined. In Ukhila, the rate of 50 for the village water collections was somewhat lower than that for the corresponding paddy fields.

The intensity of the larval occurrence of the different carrier species, recorded from the areas studied, can be well followed in the accompanying charts (Charts III, IV and V). It becomes evident from these charts that *A. varuna*, a species known to be a natural carrier (Iyengar, 1928), was much more prevalent (Charts III and IV). These charts further show that the peak of *A. varuna* breeding in the villages is usually attained in the month of September, and that there is another rise in the cold weather, usually in December; in the paddy fields the peak may be attained at about the same time. *A. aconitus* has two peaks in the village collections, the first rise being noted in the months of February and March, and the other one towards the end of the year, that is to say in December. As no water remains on the paddy fields in the months of February to June, the first rise in such situations is absent.

A. philippinensis, another species known to be infected in nature, is represented, but only in a small proportion as compared to the other species (Chart V). In whatever number they may be existing, this species has its maximum breeding in the villages during the monsoon periods, i.e., in July and August. Very few larvæ could be obtained from the paddy fields, and the curve for the *philippinensis* breeding in these does not exhibit any obvious deflection. The chart also shows the entire absence of the species from the paddy fields in 1934, up to date.

It becomes at once apparent from the same chart that *A. annularis* breeding is most abundant during the months of April and May in the villages before the commencement of the monsoon rains. In the paddy fields, however, the height of breeding for this species is usually reached in January, towards the approach of the dry season, and, as such, the species may not be of much importance in the incidence of malaria in this part of the country. Evidently long stretches of water surfaces, as are found during the monsoon or heavy rains, are not suitable for the breeding of the species to any great extent.

The species, *A. ramsayi*, has a very variable breeding season as exemplified in Chart V. There are several sharp rises in the course of its breeding. In the villages the first increase takes place in July or August, there may be another rise sometime in September or October, and yet another generally in December. The breeding of the species is almost undetectable during the dry season from February to April. The breeding in the paddy fields is not so very appreciable, except in the month of September.

I have already referred to the scanty prevalence of the *philippinensis* larvæ in the present investigation; it is worth examining the extent to which the paddy fields and the village water collections are infested with these larvæ. This has been depicted in Chart VI, where the average number of water collections found infested with this species every month has been plotted. The species thus occurred in the paddy fields of Sonarpur and Ukhila, but of course it only formed about 0.4 and 0.3 per cent respectively of the total larval catch in the two villages.

The average rate of actual infestation every month in the breeding places of the areas under observation has been represented graphically in Chart VII*.

* In Sitola the total number of paddy fields under observation nearly equalled that of the village water collections, that is to say 50 against 65 of the latter. But in Sonarpur and Ukhila the breeding places encountered in the villages much exceeded in number those in paddy-fields.

Figure 1 consists of six stacked bar charts arranged in three pairs, one pair for each village: Sonarpur, Ukhila, and Sitola. Each pair compares the land use distribution in the 'VILLAGE' area versus the 'PADDY FIELD' area. The Y-axis represents the percentage from 0 to 100. The bars are filled with various patterns to represent different land use types. For example, in Sonarpur, the Village bar shows a large cross-hatched area at the bottom (approx. 38%), while the Paddy Field bar shows a large cross-hatched area at the bottom (approx. 56%). In Ukhila, the Village bar has a large cross-hatched area (approx. 33%), and the Paddy Field bar has a large cross-hatched area (approx. 50%). In Sitola, the Village bar has a large cross-hatched area (approx. 19%), and the Paddy Field bar has a large cross-hatched area (approx. 46%).

Bar chart showing the relative prevalence of *Anopheles* larvae per 10 standardized samples in four villages: Sonapur, Ukhila, and Sitpa. Each village has two bars: one for the village and one for the port field. The Y-axis ranges from 0 to 60. The bars are stacked with various patterns representing different *Anopheles* species.

Village	Location	Species	Prevalence (approx.)
Sonapur	Village	<i>An. stephensi</i>	24
		<i>An. gambiae</i>	10
	Port Field	<i>An. stephensi</i>	27
		<i>An. gambiae</i>	16
Ukhila	Village	<i>An. stephensi</i>	18
		<i>An. gambiae</i>	16
	Port Field	<i>An. stephensi</i>	24
		<i>An. gambiae</i>	28
Sitpa	Village	<i>An. stephensi</i>	8
		<i>An. gambiae</i>	12
	Port Field	<i>An. stephensi</i>	20
		<i>An. gambiae</i>	21

CHART III.

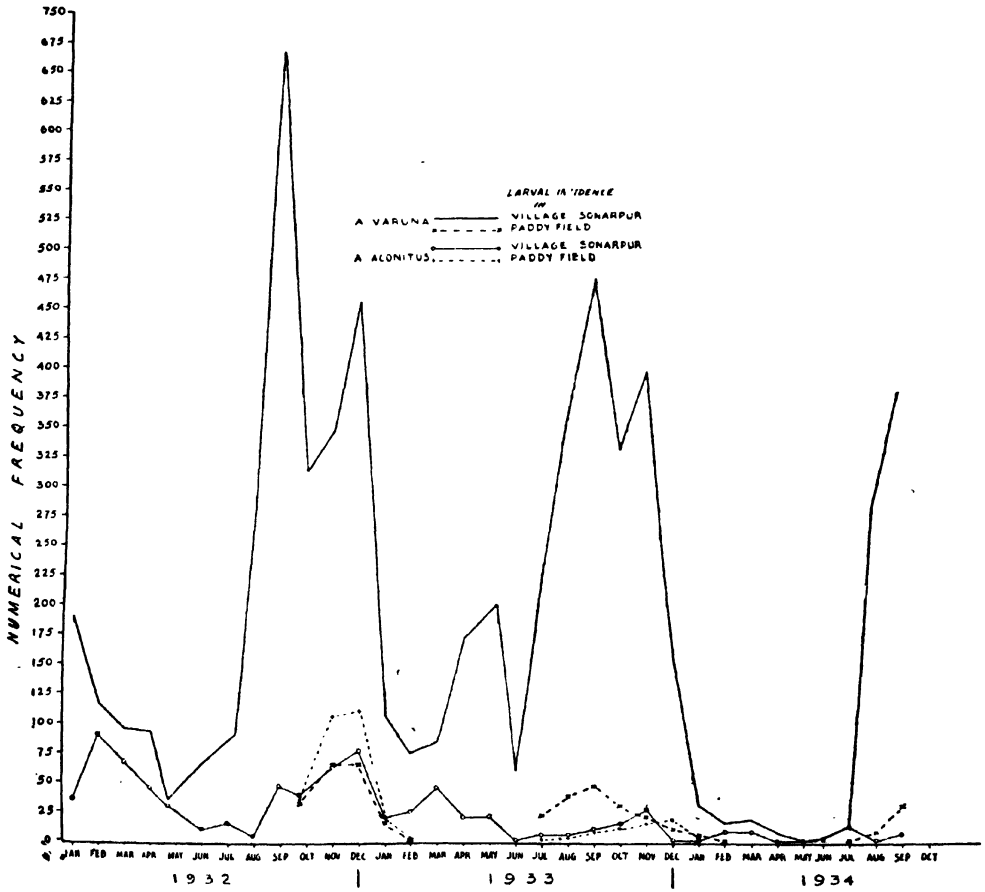


CHART IV.

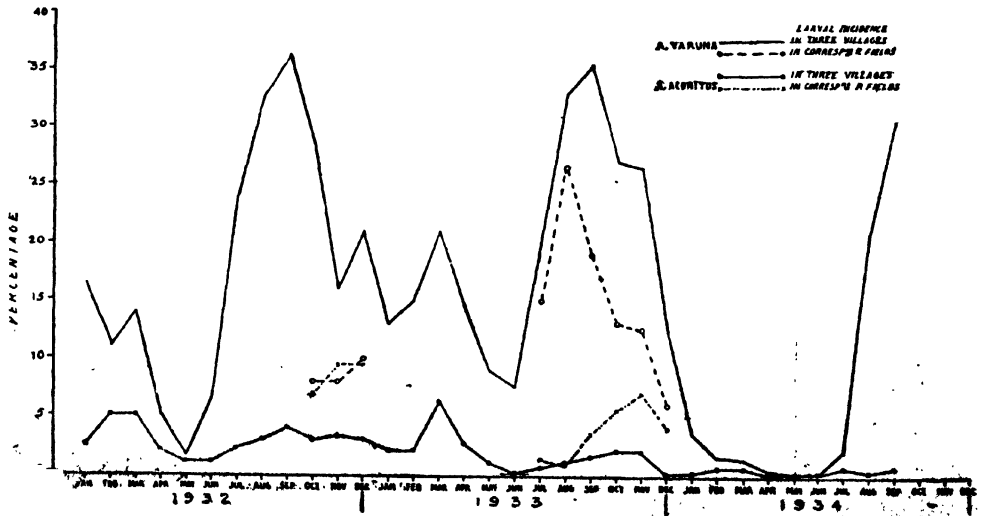


TABLE III.
Monthly spleen indices of the villages studied.

Year.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.
1. SONARPUR.												
1932 ..	8.3	8.9	9.0	9.5	10.5	10.5	9.5	9.5	9.5	10.5	9.6	9.6
1933 ..	9.3	9.4	10.4	10.7	11.5	11.5	11.3	11.3	12.3	12.6	11.1	10.0
1934 ..	9.3	8.2	8.2	8.9	9.1	9.9	11.5	12.3	12.3	12.2
2. UKHILA.												
1932 ..	48.4	50.0	50.0	51.0	52.6	53.2	52.6	52.6	52.6	..	52.6	48.8
1933 ..	48.8	48.8	51.0	51.0	51.6	53.5	54.1	51.5	..	50.0	46.8	46.6
1934 ..	47.1	46.5	42.1	42.2	43.1	42.5	46.9	46.9	46.9	47.7
3. SITOLA.												
1932 ..	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1933 ..	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1934 ..	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Note.—Children examined were about 200 in Sonarpur, 100 in Ukhila and 50 in Sitola.

Although we have shown earlier that the larval rate was highest at Sonarpur, it will now be seen that, in number of actual infestations of the breeding places, Ukhila stood at the top. Every water collection in the latter village, without any exception, was found to contain Anopheline larvæ during the height of the mosquito-breeding season in September, and 50 per cent infestation in May was the lowest that could be found in the village. Incidentally, it may be mentioned here that malaria is more rife in this village as compared with the neighbouring villages, the spleen rate here being about 50 per cent (Table III)*.

Chart VII also shows that in the paddy fields the breeding of Anophelines begins in July and usually reaches its maximum during September, when 70 to 80 per cent of the paddy fields are affected. The breeding then wanes, reaching its lowest towards the end of the cold weather, say in the month of January, when one-third of the total number of paddy fields show the presence of Anopheline larvæ. No more breeding is possible after this as the fields get dried up.

CLIMATIC EFFECT.

The total bi-monthly rainfall at Sonarpur, for the entire period the investigation was carried on, has been graphically represented in Chart VIII. The mean maximum and minimum temperatures and mean humidity month by month for the period are shown in Table IV. From a study of these figures it will be seen that the rainfall in the area is heaviest in July or August, this corresponds to the period of highest atmospheric humidity. The months from March to May appear to be the hottest period of the year.

The climatic factors thus distinctly indicate that the species *A. varuna* greatly depends on heavy rainfall and consequently high humidity for its maximum breeding, which usually takes place somewhere about September. *A. annularis* on the other hand has its maximum paddy-field breeding in January, at a time when the temperature and humidity remain low towards the close of the harvesting period. *A. philippinensis*, however, may be noticed more frequently in the paddy fields towards the end of the rainy season.

DISCUSSION.

Very interesting findings have been arrived at from this study as regards the importance of the paddy-field breeding of Anophelines in the vicinity of Bengal villages. The intensity of breeding varies in the different localities. There may be paddy fields, as in Ukhila, where the rate of breeding slightly exceeds that in the other types of breeding places within the village. Secondly, there may be places like Sitola, where the breeding in the paddy fields attains almost the same level as that in the village proper. Thirdly, there may be places like Sonarpur, where the breeding in the paddy fields is at a much lower level than that in the other forms of water collections in the village. This peculiarity in the breeding habits of the Anophelines, explains the existence of

*The village is situated on a high level with very little natural drainage. This gives a better chance for the mosquitoes to breed. Also the density of the carrier species here is above that of the neighbouring areas considered. These conditions possibly tend to make the place malarious.

CHART V.

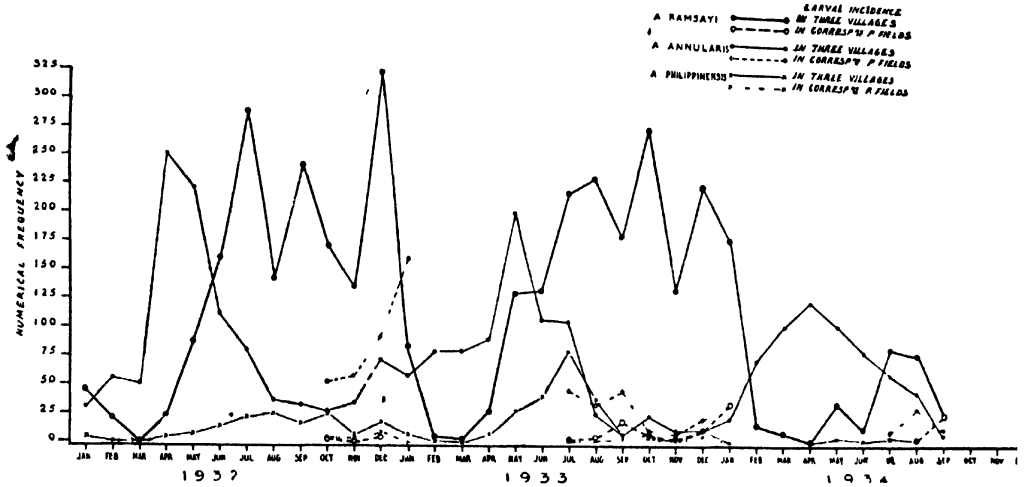


CHART VI.

RATE OF A. PHILIPPINENSIS INFESTATION

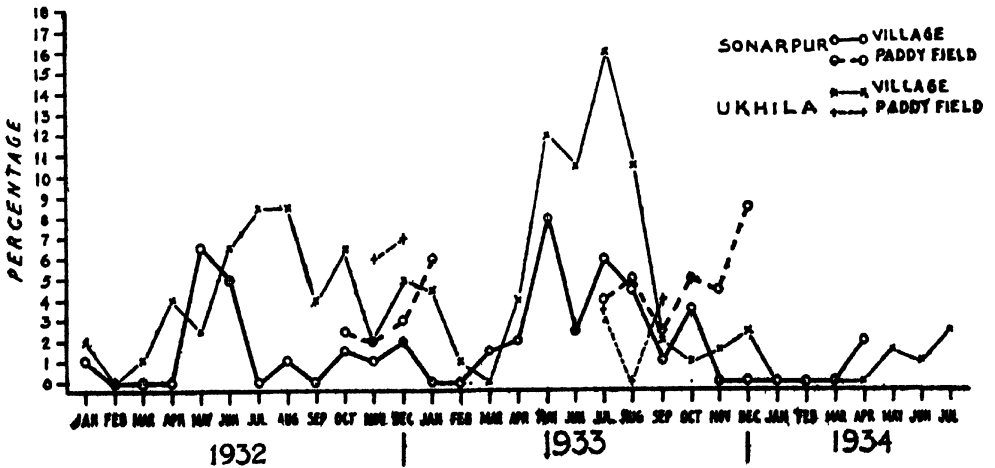


CHART VII.

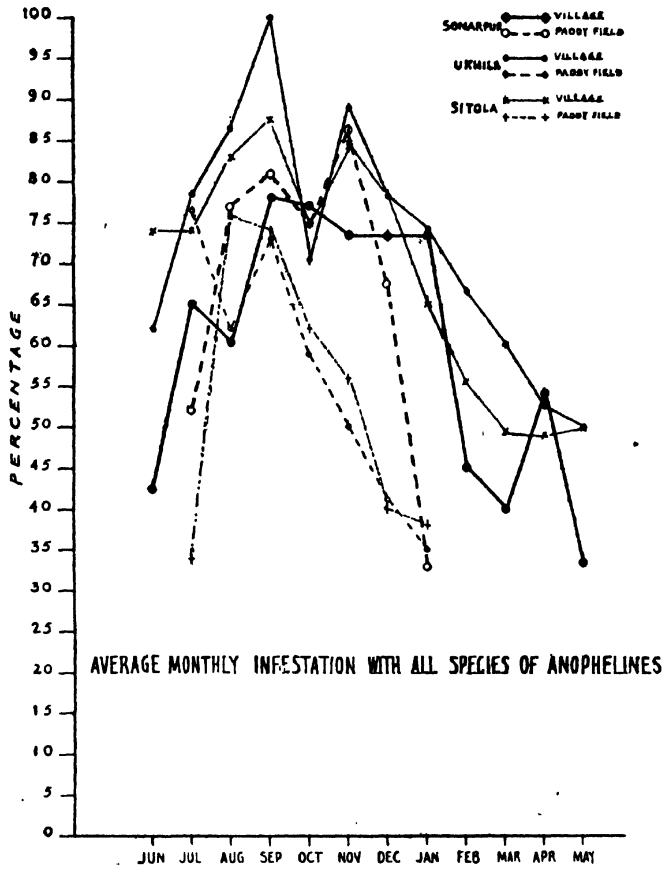
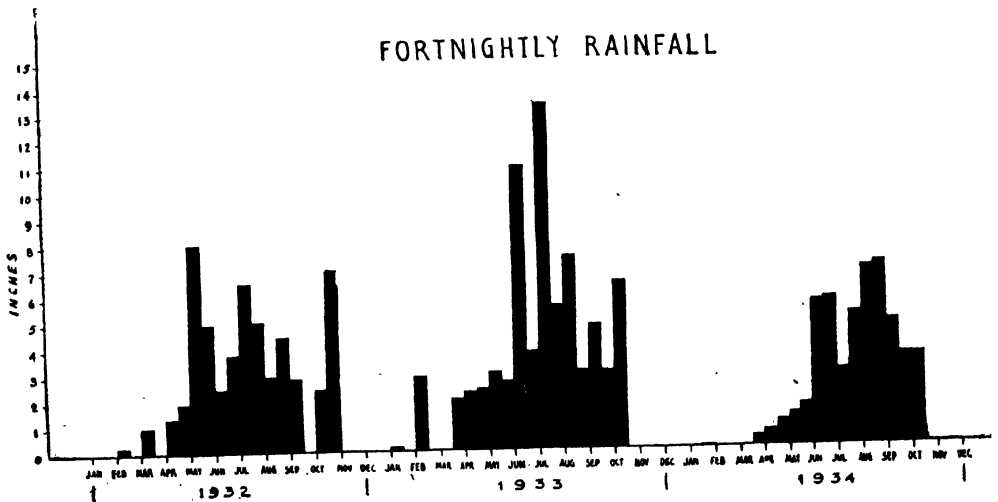


CHART VIII.



some apparently conflicting views on the subject of the dangers of rice cultivation. The scarcity of Anophelines noted by Senior White (1920) in the paddy fields of Ceylon, therefore, does not appear unnatural, although Carter (1927)

TABLE IV.
Meteorological observations.
(Sonarpur Field Malaria Laboratory.)

Years.	Months.	TEMPERATURE.		HUMIDITY. PER CENT.	RAINFALL.		Totals.
		Max.	Min.	Mean.	1st half.	2nd half.	
1932.	January ..	85.0	57.0	52
	February ..	90.0	57.0	48	..	0.2	0.2
	March ..	98.0	67.0	48	..	0.1	0.1
	April ..	101.0	73.0	74	..	1.3	1.3
	May ..	99.0	71.0	73	1.58	8.0	9.5
	June ..	96.0	76.0	84	4.9	2.49	7.3
	July ..	91.0	75.0	87	3.74	6.56	10.3
	August ..	92.0	78.0	88	5.0	2.85	7.8
	September ..	93.0	79.0	84	4.48	2.74	7.2
	October ..	94.0	73.0	76	..	2.49	2.49
	November ..	90.0	64.0	83	6.98	..	6.98
	December ..	83.0	60.0	66
1933.	January ..	84.0	53.0	61	..	0.15	0.15
	February ..	89.0	58.0	56	..	2.83	2.83
	March ..	97.0	63.0	41
	April ..	98.0	73.0	65	1.9	2.24	4.1
	May ..	98.0	72.0	66	2.4	2.98	5.3
	June ..	96.0	77.0	84	2.69	10.92	13.6
	July ..	89.0	75.0	91	3.77	13.33	17.1
	August ..	90.0	75.0	87	5.51	7.44	12.9
	September ..	93.0	78.0	84	2.94	4.73	7.6
	October ..	92.0	73.0	72	3.1	6.45	9.5
	November ..	88.0	64.0	67
	December ..	81.0	58.0	50
1934.	January ..	86.0	51.0	43
	February ..	92.0	53.0	42	..	0.03	0.03
	March ..	100.0	67.0	34
	April ..	98.0	72.0	49	0.45	0.67	1.1
	May ..	101.0	73.0	47	1.1	1.25	2.3
	June ..	96.0	74.0	76	1.62	5.61	7.2
	July ..	92.0	75.0	87	5.65	2.89	8.5
	August ..	91.0	76.0	83	5.17	6.82	11.9
	September ..	92.0	78.0	80	7.0	4.75	11.7
	October	65	3.54	3.55	7.0

a few years later, working in the same country but in a different part, obtained opposite findings. Nevertheless, the latter author at the same time made it clear that, in spite of the abundance of Anopheline larvæ, the dangerous species prevailed in low numbers only in the rice fields. My observations also show that the carrier species have different predilections in the choice of breeding

places. This is especially noticeable in the case of *A. philippinensis*; while this species breeds in the paddy fields of Sonarpur and Ukhila, it is strikingly absent from those of Sitola.

It does not appear that there is any direct correlation between the malariousness of a place and rice cultivation in lower Bengal, since, although rice is grown almost to the same extent in each of the three villages studied, Ukhila only can be said to be malarious with its spleen rate in the neighbourhood of 50 per cent, while Sitola is very healthy, the spleen rate being nil in this village. Sonarpur has a spleen rate approximating 10 per cent. This lack of correlation has also been stressed by observers like Kendrick (1914), Carter (1927) and Gill (1930).

Carter (1927) has rightly pointed out that the cultivation of rice, however scientifically done, would always create conditions suitable for mosquito breeding, but this does not mean that the cultivation must necessarily be associated with malaria. My observations fully corroborate this statement. *Every area of cultivation must be judged on its own merits.* There would always be places in every region where, in spite of intensive cultivation of rice, the place remains free of malaria. Observers like Kendrick (1914) and Gill (1930) also hold similar views. Both these authors state that rice cultivation by itself cannot be responsible for malaria, but when other factors come in, malaria may appear. These factors according to Kendrick (1914) lie in irrigation and shade. I do not find any such obvious factors determining the abundance or scarcity of Anophelines in the rice fields I studied, but this appears to depend on the physical factors prevailing in natural waters, or, in other words, on the composition of water as found by Watson (1915). The physical factors like the salinity and the saline ammonia, as already pointed out by Senior White (1928), probably involve conditions which determine in an indirect way the malarious condition of a place. Senior White (1928) observed that saline ammonia, except in the case of *rossi*-group, is inhibitory to mosquito breeding, if this content exceeds one part per million. In none of the paddy fields studied by me was this limit ever exceeded. If we accept this view, the presence of fewer Anophelines in Sitola can be thus explained, as being due to the comparatively high salinity of the water, because the paddy fields are occasionally flooded with saline water from the adjacent Salt Lake area.

Sur (1931) considers the rice fields as mainly concerned in causing the production of *A. philippinensis* in an area. Contrary to this observation, Bose (1934) found the rice fields free from this species. It is difficult for me to reconcile the statement of the latter author with my findings. Except for Sitola, where the rice fields possess high salinity, I have observed the occurrence of *A. philippinensis* in paddy fields quite often, although in numerical frequency or in proportionate distribution the species never attains a high level. However small the numbers may be, in the height of the paddy-growing season in 1933, in one village alone, *viz.*, Sonarpur, nearly 8 per cent of the rice fields showed the presence of this species, so that it would be wrong to suggest that the paddy fields do not breed *A. philippinensis*.

SUMMARY.

1. The intensity of Anopheline breeding in the paddy fields of Bengal villages varies in different localities. It is not always that the amount of

breeding in these fields is greater than the breeding of Anophelines in other types of water collections in the villages.

2. The breeding in the paddy fields commences in July and ends in January following. The maximum breeding, however, takes place in September.

3. The presence of paddy fields in the vicinity of villages does not always signify that the place will be malarious, nor do the paddy fields always breed carrier species of *Anopheles*.

4. The species noted from the different paddy fields are—*A. hyrcanus* var. *nigerrimus*, *A. barbirostris*, *A. subpictus*, *A. vagus*, *A. varuna*, *A. aconitus*, *A. ramsayi*, *A. annularis*, *A. philippinensis*, *A. pallidus* and *A. tessellatus*. Of these *A. varuna*, *A. annularis* and *A. philippinensis* have been reported to be infected in nature in Bengal.

5. The species, *A. pallidus* and *A. tessellatus*, occur only very rarely, and *A. hyrcanus* var. *nigerrimus* comprises nearly 50 per cent of the total catch.

6. *A. culicifacies* is not found to breed in the rice fields studied. If the salinity of the accumulated water be high, *A. philippinensis* also does not breed in such situations.

7. Although *A. philippinensis* may occur in the paddy fields, the numerical incidence of the species is rather low. The rate of breeding of *A. annularis* is usually greater in the paddy fields as compared to its breeding in the village water collections.

8. Long continuous stretches of water, or heavy rains, are not suitable to the breeding of *A. annularis*, the maximum breeding of which occurs only towards the close of the paddy-growing season, i.e., in January. The mean maximum temperature at this period is found to be about 85°F., and the mean minimum drops to about 54°F., when the humidity is in the neighbourhood of 50 per cent.

9. The height of the breeding of *A. varuna* which takes place about the month of September closely follows the month of maximum rainfall. The mean maximum temperature at this time of the year is about 93°F. and the mean minimum 78°F. with 84 per cent mean humidity.

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MALARIA IN SIND.

Part XIII.

MALARIA IN HALA TALUKA, HYDERABAD DISTRICT, AND IN KANDIARO, SINJHORO AND SHAHDADPUR TALUKAS, NAWABSHAH DISTRICT.

BY

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I. MALARIA IN HALA TALUKA, HYDERABAD DISTRICT.

AN account of malaria investigations in Guni, Dero Mahbat, Tando Bago and Badin Talukas of Hyderabad District was given in Part I of this series (Covell and Baily, 1930). These talukas form the Guni Division of the district, and the survey was made in September and October 1928. The observations now described were made in Hala Taluka, in the Hala Division of the district, in July 1928. Details regarding the physical features, climate, cultivation and irrigation in Hyderabad District, and also an account of epidemic malaria in the district have been given in our previous paper referred to above; and will not be repeated here.

Hala Taluka is situated on the left bank of the Indus, and has an area of 503 square miles, with a population of 113,500 (in 1931). It is at high level compared with other parts of the district, and is irregular in shape, narrowing in the middle to barely 7 miles in breadth. The cultivation is mainly that of dry crops, the chief of these being *bajra*, tobacco and cotton. About 80 per cent of the cultivation was by lift irrigation prior to the operation of the Lloyd Barrage Scheme. The observations were carried out in 10 villages, all of which

are situated within 8 miles of the River Indus. The results of spleen and blood examinations carried out in these villages are given in Tables I and II.

CHANGRO is a village with about 300 inhabitants, situated in the midst of dry cultivation, 20 miles south-east of Hala, on the bank of the Khalik Wah. This canal flows within 100 yards of the northern border of the village. The subsoil water level was 27 feet below ground level. The spleen rate was 50 per cent (50 observations), and the average enlarged spleen measured 9.7 cm. The parasite rate was 22 per cent (50 observations, M. T., 11).

BHAAO KHAN is a village with a population of about 750, situated in the midst of dry cultivation, 19 miles south-east of Hala. The subsoil water level was 26 feet. The spleen rate was 68 per cent (70 observations) and the average enlarged spleen was 7.3 cm. The parasite rate was 27 per cent (70 observations, 18 M. T., 1 Q.). Crescents were observed in one case.

UBRA is a village with about 100 inhabitants, situated in the midst of dry cultivation, 18 miles south-east of Hala. A canal distributary flows within 400 yards of the village. The subsoil water level was 31 feet. The spleen rate was 44 per cent (25 observations), and the average enlarged spleen was 8.0 cm. The parasite rate was 12 per cent (25 observations, 2 M. T., 1 Q.). Crescents were observed in both the malignant tertian cases.

ODERO LAL is a village with a population of about 700, situated in the midst of dry cultivation, 16 miles south-east of Hala. The subsoil water level was 27 feet. The spleen rate was 7 per cent (60 observations) and the average enlarged spleen was 9.2 cm. The parasite rate was 8 per cent (60 observations, M. T., 5). This village was situated on high ground, and there were no breeding places in the immediate vicinity.

PINIA KHEDRI is a village with about 400 inhabitants, situated in the midst of dry cultivation, 16 miles south of Hala. The subsoil water level was 27 feet. The spleen rate was 16 per cent (86 observations) and the average enlarged spleen was 8.8 cm. The parasite rate was 15 per cent (41 observations, M. T., 6). Crescents were observed in one case.

SHER MOHAMED THORA is a village with a population of about 1,000, situated in the midst of dry cultivation, 18 miles south of Hala. The subsoil water level was 22 feet. The spleen rate was 3 per cent (134 observations), and the average enlarged spleen was 8.8 cm. Only five children were examined for malaria parasites, one of these showing a malignant tertian infection.

MATIARI is a village with about 4,500 inhabitants, situated on the bank of the Khair Wah in the midst of dry cultivation, 22 miles south of Hala. The subsoil water level was 25 feet. The spleen rate was 27 per cent (150 observations), and the average enlarged spleen was 8.6 cm. The parasite rate was 18 per cent (56 observations, 10 M. T., 1 Q.). Crescents were observed in one case.

PORATH is a village with a population of about 200, situated on the bank of the Khair Wah, 20 miles south of Hala, and about two miles from the River Indus. The subsoil water level was 14 feet. The spleen rate was 31 per cent (45 observations), and the average enlarged spleen was 9.4 cm. The parasite rate was 13 per cent (45 observations, 3 M. T., 3 B. T.).

SHEKHAT is a village with about 700 inhabitants, situated 2 miles east of the Indus, and 18 miles south of Hala, in the midst of dry cultivation: The

subsoil water level was 13 feet. The spleen rate was 55 per cent (75 observations), and the average enlarged spleen was 8.5 cm. The parasite rate was 13 per cent (75 observations, 8 M. T., 3 B. T.). Crescents were observed in one case.

HALA, the headquarters of the taluka, has a population of about 6,000, and is situated in the midst of dry cultivation, 6 miles east of the Indus, and 14 miles south-west of Shahdadpur. There were a number of irrigation wells on the outskirts of the town, from some of which larvæ of *A. culicifacies* were collected. The spleen rate was 5 per cent (112 observations), and the average enlarged spleen was 9.0 cm. The parasite rate was 10 per cent (100 observations, 9 M. T., 1 B. T.).

SUMMARY.

The spleen rates varied very considerably in different villages, the highest rates being generally found in those nearest to the Indus, where the subsoil water level was also higher. Most of the villages were surrounded by excavations of various sizes, fed by canal distributaries, and used for irrigation purposes. They are filled towards the end of the irrigation season for winter cultivation, and no doubt act as excellent breeding places for *A. culicifacies*. Three of the 10 villages yielded spleen rates of 50 per cent or more, and there is no doubt that the amount of endemic malaria in the taluka is considerable. The combined spleen rate of 24 per cent is certainly too low, being brought down by the comparatively low rates in the larger villages, where more children were available for examination.

II. MALARIA IN SHAHDADPUR, SINJHORO AND KANDIARO TALUKAS, NAWABSHAH DISTRICT.

An account of malaria investigations in Nausharo, Moro, Sakrand, Nawabshah and Shahdadpur Talukas of Nawabshah District was given in Part IV of this series (Covell and Baily, 1931). These observations were carried out from 22nd December, 1928, to 17th January, 1929, i.e., at the end of the malaria season. In the present paper further investigations made in Shahdadpur Taluka in February 1932 are described. Observations were made in Sinjhor Taluka at the same period, i.e., just before the commencement of the operation of the Lloyd Barrage Scheme, whilst the survey in Kandiaro Taluka was made in March 1933, nine months after the new system of irrigation had commenced. Details regarding the physical features, climate, cultivation and irrigation in Nawabshah District, and also an account of epidemic malaria in the district, have been given in our previous paper referred to above, and will not be repeated here.

1. SHAHDADPUR TALUKA.

This taluka lies immediately to the west of Sinjhor Taluka, and is separated from the Indus by the talukas of Sakrand and Hala. It covers an area of 644 square miles, and has a population of 103,200 (in 1931). The taluka lies at a high level, and produces the best cotton in the district, and also good *bajra* and tobacco crops. The results of spleen examinations carried out in 14 villages in the taluka are given in Table III.

AHMAD ABAD is a village with about 350 inhabitants, situated in the midst of dry cultivation, half a mile west of Jamrao Canal, and 22 miles north-east of Shahdadpur. The subsoil water level in the wells varied from 20 to 15 feet. The spleen rate was 66 per cent (35 observations), and the average enlarged spleen was 8·6 cm.

SHAHPUR CHAKAR is a village with about 1,500 inhabitants, situated in the midst of dry cultivation, 18 miles north-east of Shahdadpur. A canal distributary ran through the middle of the village, but the villagers stated that this would shortly be closed, and irrigation would be carried out from a canal flowing about 3 miles to the north. The subsoil water level was 17 feet. The spleen rate was 61 per cent (142 observations), and the average enlarged spleen was 10·6 cm.

UMAR DAHRI is a village with a population of about 150, situated in the midst of dry cultivation, 17 miles north of Shahdadpur. The subsoil water level was 15 feet. The spleen rate was 45 per cent (40 observations), and the average enlarged spleen was 9·8 cm.

ALI MURAD is a hamlet with about 50 inhabitants, situated in the midst of dry cultivation, 22 miles north-east of Shahdadpur. The subsoil water level was 15 feet. The spleen rate was 30 per cent (20 observations), and the average enlarged spleen was 9·8 cm.

LANDI is a village with about 100 inhabitants, and is situated in the midst of rice cultivation, 8 miles east of Shahdadpur. The subsoil water level was 11 feet, and there was a canal distributary within 200 yards of the village. The spleen rate was 35 per cent (26 observations), and the average enlarged spleen was 11·4 cm.

DUTHRO is a village with a population of about 500, situated in the midst of rice cultivation, 6 miles east of Shahdadpur. A canal distributary flows within 400 yards of the village. The subsoil water level was 11 feet. The spleen rate was 66 per cent (100 observations), and the average enlarged spleen was 9·2 cm.

SHAHDADPUR, the headquarters of the taluka, has a population of about 7,500, and is situated on the bank of the Raj Wah, four miles west of Jamrao Canal. There is rice cultivation in the neighbourhood, but none within a mile of the town. The subsoil water level in various wells varied from 17 to 20 feet. The spleen rate was 19 per cent (347 observations), and the average enlarged spleen was 9·2 cm. The parasite rate was 20 per cent (25 observations, 5 M. T.).

MALDASI is a village with about 500 inhabitants, situated in the midst of dry cultivation, 7 miles west of Shahdadpur. The Rohri Canal passes within 100 yards of the village on the western side, but the land around the village is irrigated from the Marak Wah. The subsoil water level was 18 feet. The spleen rate was 29 per cent (124 observations), and the average enlarged spleen was 9·2 cm.

LALL KHAN JALALANI is a village with about 300 inhabitants, situated in the midst of dry cultivation, 6 miles north-west of Shahdadpur. A canal distributary flows within 400 yards of the village. The subsoil water level was

19 feet. The spleen rate was 27 per cent (33 observations), and the average enlarged spleen was 9.4 cm.

TALPUR is a village with a population of about 400, situated in the midst of dry cultivation, 5 miles north-west of Shahdadpur. A canal distributary passes within 400 yards of the village on the southern side. The subsoil water level was 25 feet. The spleen rate was 22 per cent (60 observations), and the average enlarged spleen was 11.0 cm.

UMAR KHAN LAKHO is a hamlet with about 60 inhabitants, situated in the midst of dry cultivation, 3 miles north-west of Shahdadpur. There was a certain amount of rice cultivation about half a mile from the village. The subsoil water level was 20 feet. The spleen rate was 24 per cent (25 observations), and the average enlarged spleen was 9.7 cm.

SUTIARI I is a village with a population of about 400, situated in the midst of dry cultivation, 5 miles south of Shahdadpur. The subsoil water level was 21 feet. The spleen rate was 40 per cent (77 observations), and the average enlarged spleen was 10.3 cm.

SUTIARI II is a village with about 200 inhabitants, situated 7 miles south of Shahdadpur. The surrounding cultivation is mainly dry-crop, but a certain amount of rice is grown within 400 yards of the village. The subsoil water level was 20 feet. The spleen rate was 64 per cent (39 observations), and the average enlarged spleen was 8.0 cm.

RAJO MARI is a village with a population of about 550, situated 7 miles south of Shahdadpur. A canal distributary runs close by the village, but this was dry at the time of the survey. The subsoil water level was 20 feet. The spleen rate was 17 per cent (80 observations), and the average enlarged spleen was 11.0 cm.

2. SINJHORO TALUKA.

This taluka lies immediately to the east of Shahdadpur Taluka, and covers an area of 475 square miles, with a population of about 48,200 (in 1931). It is irrigated by the Jamrao Canal and its branches, the chief crops being cotton and wheat. About 70 per cent of the cultivation was by lift irrigation before the operation of the Lloyd Barrage Scheme. During the course of the survey, which took place in February 1932, observations were made in 16 villages. The results of spleen examinations made in this taluka are given in Table IV.

HAJI BUDHAL KHAN is a village with about 150 inhabitants, situated 12 miles north-west of Sinjhor, in the midst of dry cultivation. The Jamrao Canal flows about 400 yards from the west of the village. The subsoil water level was 15 feet. The spleen rate was 38 per cent (13 observations only), and the average enlarged spleen was 9.6 cm.

QAIM KHAN is a village with about 120 inhabitants, situated 12 miles north-east of Sinjhor, in the midst of dry cultivation. There is a canal distributary within 400 yards of the village. The subsoil water level in a well about 3 miles from the village was 16 feet. The spleen rate was 17 per cent (6 observations only).

HARDASMAL JO GOT is a village with a population of about 50, situated in the midst of dry cultivation, 12 miles north-west of Sinjhor. The subsoil water

level was 13 feet. The spleen rate was 55 per cent (9 observations only), and the average enlarged spleen was 8.0 cm.

DEH No. 16 is a village with about 150 inhabitants, situated in the midst of dry cultivation, 12 miles north of Sinjhor. The subsoil water level was 13 feet. The inhabitants were Sikh colonists originating from the district of Amritsar. The spleen rate was 74 per cent (27 observations), and the average enlarged spleen was 8.7 cm.

DEH No. 20 is a village with about 100 inhabitants, situated 14 miles north-west of Sinjhor, in the midst of dry cultivation. A canal distributary flows within 400 yards of the village. The subsoil water level was 15 feet. The spleen rate was 41 per cent (22 observations), and the average enlarged spleen was 9.0 cm.

KHADRO is a village with a population of about 1,200, situated on the left bank of the Jamrao Canal, 10 miles north-west of Sinjhor, in the midst of dry cultivation. The subsoil water level was 14 feet. The spleen rate was 52 per cent (121 observations), and the average enlarged spleen was 9.6 cm. The parasite rate was 16 per cent (25 observations, 4 M. T.).

DEH No. 15 is a village with about 100 inhabitants, colonists from the Punjab, situated 15 miles north-west of Sinjhor. There is a canal distributary within 400 yards of the village. The subsoil water level was 11½ feet. The spleen rate was 57 per cent (30 observations), and the average enlarged spleen was 8.5 cm.

DEH No. 12 is a village with a population of about 150, situated 15 miles north-west of Sinjhor, in the midst of dry cultivation. There was no well, so that the subsoil water level could not be measured. The spleen rate was 50 per cent (42 observations), and the average enlarged spleen was 10 cm.

PRETAM ABAD is a village with about 250 inhabitants, situated 15 miles north-west of Sinjhor, in the midst of dry cultivation. The Jamrao Canal runs within a mile of the village, and a distributary originating from it passes close by. The subsoil water level was 16 feet. The spleen rate was 61 per cent (44 observations), and the average enlarged spleen was 9.5 cm.

ISMAILPUR is a village with a population of about 700, situated in the midst of dry cultivation, 7 miles north-west of Sinjhor. A canal distributary runs through the village. The subsoil water level was 21 feet. The spleen rate was 32 per cent (60 observations), and the average enlarged spleen was 9.6 cm.

DEH No. 25 is a village with about 60 inhabitants, situated in the midst of dry cultivation, 9 miles north-west of Sinjhor. The subsoil water level was 20 feet. The spleen rate was 24 per cent (29 observations), and the average enlarged spleen was 12.3 cm.

DEH No. 29 is a village with a population of about 120, situated 5 miles north-west of Sinjhor, in the midst of dry cultivation. As there was no well, the subsoil water level could not be observed. The spleen rate was 26 per cent (23 observations), and the average enlarged spleen was 10.0 cm.

SINJHORO, the headquarters of the taluka, is situated on the Mirpurkhas-Khadro railway, 6 miles east of Jamrao Canal and 8 miles west of Sanghar. The population is about 700, and it is surrounded by dry cultivation. The

subsoil water level was 17 feet. The spleen rate was 68 per cent (47 observations), and the average enlarged spleen was 9.4 cm. The parasite rate was 16 per cent (25 observations, 4 M. T.).

TILUKHAN LAGHARI is a village with about 200 inhabitants, situated in the midst of dry cultivation, 2 miles west of Sinjhor. There was no well, so that it was not possible to observe the subsoil water level. The spleen rate was 50 per cent (32 observations), and the average enlarged spleen was 10.5 cm.

KACHI Jo Gor is a village with a population of about 350, situated in the midst of dry cultivation, 4 miles west of Sinjhor. The subsoil water level was 20 feet. The spleen rate was 36 per cent (75 observations), and the average enlarged spleen was 8.3 cm.

SAID ALI MOHAMED is a village with about 100 inhabitants, situated in the midst of dry cultivation, 5 miles west of Sinjhor. The subsoil water level could not be observed, as there was no well. The spleen rate was 29 per cent (24 observations), and the average enlarged spleen was 8.6 cm.

3. KANDIARO TALUKA.

This taluka is situated at the extreme north of Nawabshah District, and covers an area of 405 square miles along the left bank of the Indus, with a population of 80,800 (in 1931). The river frontage to the width of two to three miles is covered by a belt of forest, which protects the taluka from floods. The chief summer crops are *juari* and *bajra*, and the chief winter crop is wheat. About 70 per cent of the cultivation was by lift irrigation, before the commencement of the operation of the Lloyd Barrage Scheme. Under this scheme many of the original canal distributaries have been closed and replaced by new ones. The survey took place in March 1933, and observations were carried out in 8 villages. The results of spleen examinations are given in Table V.

KAMAL DERO is a village with a population of about 700, situated in the midst of dry cultivation, 6 miles west of Kandiaro. The Mahesar Canal runs within 300 yards of the village on the northern aspect. During the summer months, when the Indus rises, its waters extend to within $1\frac{1}{2}$ miles of the village. The subsoil water level was 11 feet. The spleen rate was 34 per cent (142 observations), and the average enlarged spleen was 8.7 cm. The parasite rate was 6 per cent (50 observations, 3 M. T.).

MAHI JA BHAN is a village with about 150 inhabitants, situated 6 miles west of Kandiaro, in the midst of dry cultivation. The subsoil water level was 9 feet. The spleen rate was 36 per cent (25 observations), and the average enlarged spleen was 8.4 cm.

MAHESAR is a village with a population of about 250, situated in the midst of dry cultivation, 5 miles west of Kandiaro. The subsoil water level was 6 feet. The spleen rate was 35 per cent (40 observations), and the average enlarged spleen was 8.8 cm.

LAKHA is a village with about 300 inhabitants, situated 6 miles south of Kandiaro, in the midst of dry cultivation. The Nasrat Canal flows within 300 yards of the village on the eastern side. The subsoil water level was 15 feet. The spleen rate was 42 per cent (117 observations), and the average enlarged

spleen was 8.0 cm. The parasite rate was 5 per cent (40 observations, 2 M. T.). Crescents were observed in one case.

MAHABAT DERO JATOI is a village with a population of about 1,200, situated 6 miles north of Kandiaro, in the midst of dry cultivation. A branch of Naulakhi Canal, which takes off from the Rohri Canal runs within 400 yards of the village. The subsoil water level was 9 feet. The spleen rate was 58 per cent (96 observations), and the average enlarged spleen was 6.7 cm. The parasite rate was 6 per cent (50 observations, 3 M. T.). Crescents were observed in one case.

BAHNWAR is a village with about 400 inhabitants, situated 8 miles north of Kandiaro, in the midst of fruit gardens. The subsoil water level was 11 feet. The spleen rate was 50 per cent (78 observations), and the average enlarged spleen was 7.9 cm.

KANDIARO, the headquarters of the taluka, is situated 8 miles east of the Indus, and has a population of about 3,300. The village is situated on an eminence, and there are a number of irrigation wells surrounding it. The subsoil water level in these was about 15 feet, though the level in a well in the centre of the village was 33 feet. The surrounding cultivation is dry-crop. The spleen rate was 49 per cent (226 observations), and the average enlarged spleen was 7.3 cm. The parasite rate was 12 per cent (100 observations, 12 M. T.).

HALANI is a village with about 1,500 inhabitants, situated in the midst of dry cultivation, 7 miles north-east of Kandiaro. The subsoil water level was 26 feet in the village, which stands on an eminence, but only 14 feet in an irrigation well nearby. The spleen rate was 41 per cent (200 observations), and the average enlarged spleen was 7.2 cm. The parasite rate was 12 per cent (40 observations, 4 M. T., 1 B. T.).

SUMMARY.

The results of observations made in the three talukas of Nawabshah District show that there is a considerable amount of endemic malaria in each case, although the incidence of the disease is on the whole much less than in the rice-growing area on the right bank of the Indus. The combined spleen rates for each of the three talukas were 35 per cent in Shahdadpur, 47 per cent in Sinjhor, and 44 per cent in Kandiaro.

Spleen rates of 50 per cent and over were recorded in 4 villages out of 14 in Shahdadpur, 8 out of 16 in Sinjhor, and 2 out of 8 in Kandiaro. The observations in Shahdadpur and Sinjhor were made in February 1932, just before the commencement of the operation of the Lloyd Barrage Scheme. Those in Kandiaro were made in March 1933, 9 months after the Barrage operations commenced. As no observations were made in the taluka prior to this, it is not possible to say whether the opening of the Barrage Canals had any effect on the incidence of malaria during the first season of operation.

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TABLE I.
Results of spleen examinations in Hala Taluka.

Locality.	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*
Changro	50	25	50.0	9.7
Bhaoo Khan	70	47	67.7	7.3
Ubra	25	11	44.0	8.0
Odero Lal	60	4	6.7	9.2
Pinia Khedri	86	14	16.4	8.8
Sher Mohamed Thora	134	5	3.0	8.8
Matari	150	41	27.3	8.6
Porath	45	14	31.0	9.4
Shekhat	75	41	54.7	8.5
Hala	112	6	5.4	9.0
TOTAL ..	807	208	24.5	8.4

* Measurement in centimetres from apex of spleen to umbilicus.

TABLE II.
Results of blood examinations in Hala Taluka.

Locality.	Number examined.	Number with parasites.	Parasite rate.	M. T.	B. T.	Q.	Crescents.
Changro	50	11	22.0	11
Bhaoo Khan	70	19	27.0	18	..	1	1
Ubra	25	3	12.0	2	..	1	2
Odero Lal	60	5	8.3	5
Pinia Khedri	41	6	14.6	6	1
Sher Mohamed Thora	5	1	20.0	1
Matari	56	11	17.8	10	..	1	1
Porath	45	6	13.3	3	3
Shekhat	75	10	13.3	8	3	..	1
Hala	100	10	10.0	9	1
TOTAL ..	527	82	15.6	73	7	3	6

TABLE III.

Results of spleen examinations in Shahdadpur Taluka.

Locality.	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*
Ahmad Abad ..	35	23	65·7	8·6
Shahpur Chakar ..	142	87	61·3	10·6
Umar Dahri ..	40	18	45·0	9·8
Ali Murad ..	20	6	30·0	9·8
Landhi ..	26	9	34·7	11·4
Duthro ..	100	66	66·0	9·2
Shahdadpur ..	347	65	18·7	9·2
Maldasi ..	124	36	29·0	9·2
Lall Khan Jalalani ..	33	9	27·3	9·4
Talpur ..	60	13	21·7	11·0
Umar Khan Lakho ..	25	6	24·0	9·7
Sutiari I ..	77	31	40·2	10·3
Sutiari II ..	39	25	64·0	8·0
Rajo Mari ..	80	14	17·5	11·1
TOTAL ..	1,148	408	35·5	9·4

TABLE IV.

Results of spleen examinations in Sinjhor Taluka.

Locality.	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*
Haji Budhal Khan ..	13	5	38·5	9·6
Qaim Khan ..	6	1	16·7	..
Hardasmal Jo Got ..	9	5	55·6	8·0
Deh No. 16 ..	27	20	74·0	8·7
Deh No. 20 ..	22	9	40·9	9·0
Khadro ..	121	63	52·0	9·6
Deh No. 15 ..	30	17	56·7	8·5
Deh No. 12 ..	42	21	50·0	10·0
Pretam Abad ..	44	27	61·3	9·5
Ismailpur ..	60	19	31·7	9·6
Deh No. 25 ..	29	7	24·0	12·3
Deh No. 29 ..	23	6	26·0	10·0
Sinjhor ..	47	32	68·0	9·4
Tilukhan Laghari ..	32	16	50·0	10·5
Kachi Jo Got ..	75	27	36·0	8·3
Said Ali Mohamed ..	24	7	29·2	8·6
TOTAL ..	604	282	46·7	9·4

* Measurement in centimetres from apex of spleen to umbilicus.

TABLE V.

Results of spleen examinations in Kandiaro Taluka.

Locality.	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*
Kamal Dero ..	142	49	34.5	8.7
Mahi Ja Bhan ..	25	9	36.0	8.4
Mahesar ..	40	14	35.0	8.8
Lakha ..	117	49	42.0	8.0
Mahabat Dero Jatoi ..	96	56	58.3	6.7
Bahnwar ..	78	39	50.0	7.9
Kandiaro ..	226	111	49.0	7.3
Halani ..	200	82	41.0	7.2
TOTAL ..	924	409	44.3	7.6

* Measurement in centimetres from apex of spleen to umbilicus.

MALARIA IN SIND.

Part XIV.

THE SEASONAL INCIDENCE OF INFECTION WITH THE DIFFERENT SPECIES OF MALARIA PARASITE IN LARKANA.

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INTRODUCTION.

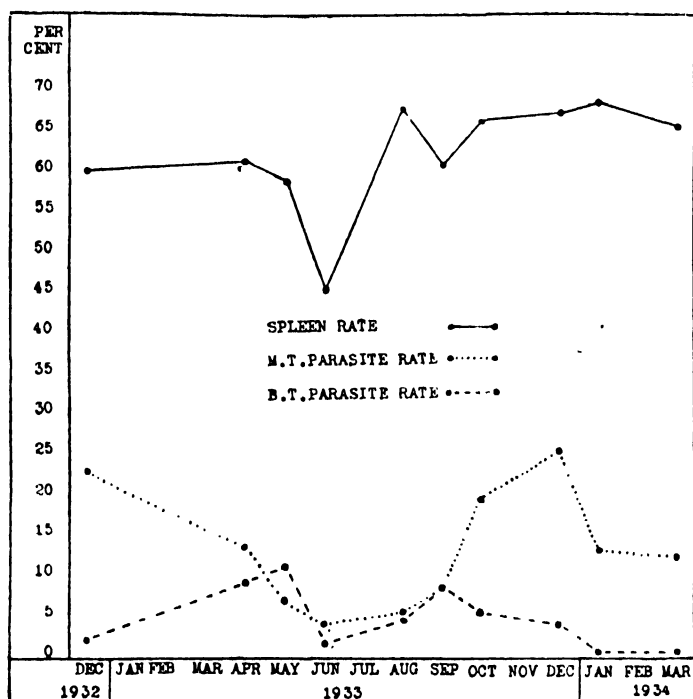
THE data which form the basis of this paper were obtained from a series of spleen and blood examinations of school children in Larkana, which is the headquarter town of Larkana District, in Northern Sind. Malaria is hyper-endemic in most parts of the district, which forms part of the great rice-growing area situated on the right bank of the Indus. The town, which has about 20,000 inhabitants, is situated on the bank of the Ghar Canal, one of the original inundation canals of Sind. This part of the canal has been cut off from the lower portion by the Central Rice Canal, one of the great new canals forming part of the Lloyd Barrage Scheme, so that it has become a stagnant backwater (Covell and Baily, 1934).

The first observations were made in December 1932, and further examinations were carried out during each month from April 1933 to March 1934, with the exception of July and November 1933 and February 1934. The children, whose ages ranged from 5 to 10 years, were scholars of four primary schools, namely, Shah Mohamed Municipal and Main Municipal Schools, and Nawa Tak and Lahari Schools. The two former are situated in the centre of the town, and the two latter in the periphery, Nawa Tak on the south-west and Lahari on the east. The results of spleen and blood examinations (Table I) show

that the incidence of malaria is considerably greater in the outskirts of the town than in the centre. The number of observations made in the central schools was nearly five times as many as in the outlying schools, so that the combined results of our observations are more representative of urban than of rural conditions.

Altogether, 4,932 spleen observations were made, of which 2,970 showed enlargement of the organ. The total number of blood films examined was 3,548, of which 573 showed parasites (M. T., 400, B. T., 156, mixed M. T. and B. T., 17).^{*} Crescents were observed in 59 cases. Enlarged spleens were measured in all cases by the method of Christophers (1924), and parasite counts were made of all positive films by the fowl-cell method of Sinton (1924).

The results of spleen and blood examinations made in each of the four schools are given in Table I. Table II gives the combined results for the four schools, whilst in Table III the data are arranged to illustrate the relation of the species of parasite and the parasite count to the size of spleen. The spleen rates and the M. T. and B. T. parasite rates recorded at the various visits are shown in the accompanying graph.



In the autumn of 1929 there was a severe regional epidemic of malaria throughout Northern Sind, as the result of which spleen rates were raised to a very high figure, often in the neighbourhood of 90 per cent, throughout Larkana

^{*} M. T. means *P. falciparum* and B. T. means *P. vivax*.

District (Covell and Baily, 1934). The only indication we have of the degree of malaria incidence in Larkana town during the inter-epidemic period is an observation of Young and Majid (1930), who recorded a spleen rate of 27 per cent among 250 children examined at the 'High School' (presumably the Main Municipal School). The various factors influencing the normal autumnal incidence of malaria in Larkana Taluka have been discussed in detail by Covell and Baily (1930).

RESULTS OF OBSERVATIONS.

With regard to the spleen rate, it will be noticed that the results were remarkably uniform throughout the period under review, with the exception of a marked drop in the figures for the month of June. The rates in the central schools dropped from 58 and 60 per cent in May to 41 and 42 per cent in June, whilst in the outlying schools (where no observations were made in May), there was a fall from 76 and 96 per cent in April to 64 and 74 per cent in June respectively. No observations were possible in July, but in August the spleen rates had risen to 64 per cent and 57 per cent in the central schools, and 84 per cent and 92 per cent in the outlying schools. The spleen rates remained at approximately the same height throughout the remainder of the period under review.

The average enlarged spleen was also at its smallest size in June (8.4 cm. for the four schools combined). It reached its greatest size in the month of October (7.0 cm.).*

The M. T. parasite rate was at its lowest in June (4 per cent for the four schools combined). It showed a gradual rise in August and September, which became more marked in October (20 per cent), reaching its peak of 26 per cent in December. It should be noted, however, that no observations were made in November, and the true peak may have been in that month.

The B. T. parasite rate differed from the M. T. rate in showing two distinct peaks during the year, one in September (9 per cent for the four schools combined), and one in May (11 per cent). In the latter month, B. T. infections were more than 50 per cent as numerous as M. T. infections, whilst in most of the other months in which examinations were carried out, M. T. infections predominated.

The crescent rate was at its highest during the period October to December.

DISCUSSION.

In a previous paper, dealing with the regional epidemic of malaria in Northern Sind (Covell and Baily, 1932), we showed that whilst *P. falciparum* was the principal malaria parasite concerned in the epidemic, there were a number of benign tertian infections acquired in its early stages. We concluded that a proportion of these cases produced attacks of malaria in the spring, these being either delayed primary attacks or late relapses. We based this

* The measurements given represent the distance in centimetres from the apex of the spleen to the umbilicus. This distance of course becomes smaller as the spleen enlarges.

conclusion on the fact that there was a marked increase in the spleen rate during the first eight months of the post-epidemic period, associated with a decrease in the size of the average enlarged spleen, and a considerable increase in the B. T. parasite rate, which had fallen to a very low figure during the latter half of the epidemic period.

We favoured the view that most of these hypothetical benign tertian attacks were delayed primary attacks, because of the decrease in the size of the average enlarged spleen, which was everywhere noticeable. Had they been chiefly relapses, we would have expected an increase, instead of a decrease, in the size of the average enlarged spleen. As against the theory that the attacks might be the result of infections acquired during the spring, we pointed out that this was unlikely, owing to the almost complete absence of the carrier species of *Anopheles* during this period, and the generally unfavourable meteorological conditions for transmission.

The observations detailed in the present paper in general lend support to the above hypothesis. The course of events is remarkably like that observed in Shikarpur during and immediately after the 1929 epidemic, except that in that year the infections (especially with *P. falciparum*) were much more numerous and more severe. In both cases there is the early rise in B. T. infections in the early autumn, followed by a steady fall, whilst the M. T. infections show a progressive rise in number until December. The rise in the crescent rate in October is also shown in both cases. The rise in the number of B. T. infections in the spring of 1933 was well marked, and the size of the average enlarged spleen was small at this period, suggesting that there were a number of fresh infections rather than relapses, as was the case after the 1929 epidemic. We have, however, no definite evidence that the spring incidence of benign tertian infections was due to delayed primary attacks, rather than to fresh infections, though we are inclined to favour the former view in this case also.

A prominent feature of our observations is the evidence pointing to the very high incidence of malaria persisting in Larkana throughout the period under review. Regional epidemics of malaria occur in Northern Sind at intervals of about 10 to 12 years. Thus there were epidemics in the years 1897, 1906, 1917 and 1929. During the years immediately following an epidemic the incidence of malaria normally decreases progressively till it reaches what is termed its inter-epidemic level. This varies greatly in different localities, being dependent on the degree in which local circumstances are favourable to the maintenance of endemic malaria. In the case of Larkana, a town with 20,000 inhabitants, we should expect that the normal incidence of malaria would be fairly low, at any rate in the central portion of the town. It has been stated above that Young and Majid (1930) recorded a spleen rate of 27 per cent among children examined in one of the schools of the town in 1927, two years prior to the epidemic. It is therefore remarkable that, five years after the epidemic, the spleen rates in March should be as high as 56 per cent among children living in the centre of the town, and 90 per cent among those living on its outskirts.

We have shown in a previous paper (Covell and Baily, 1934), that the abnormally high incidence of malaria in Larkana District in 1932 was probably due to the effects of the operation of the Lloyd Barrage Scheme. An account of the changed conditions produced by the Barrage Scheme was given in that

paper, and will not be repeated here. It is our opinion that, in the absence of any other apparent cause, the continuance of the high incidence of malaria in this district is attributable mainly, if not entirely, to the conditions produced by the operation of the Barrage Scheme.

SUMMARY.

1. The results of a series of spleen and blood examinations of school children in Larkana town during the period December 1932 to March 1934 are given in detail.

2. The number of spleen examinations was 4,932, and of blood examinations 3,548. All enlarged spleens were accurately measured, and parasite counts were made of all blood films found infected.

3. The curve of malignant tertian infections showed a single peak representing the usual autumnal epidemic of malaria. The benign tertian infection curve showed two peaks, one in the early autumn, the other in the spring.

4. It is considered that the spring rise in the number of benign tertian infections is due to primary attacks rather than to relapses. We consider it probable that a number of these attacks are due to primary infections acquired during the previous autumn.

5. In general, the course of events as regards the seasonal incidence of the different species of malaria parasite is remarkably similar to that observed in Shikarpur, during and immediately after the regional epidemic of 1929.

6. We consider that the high incidence of malaria persisting in this area five years after the last regional epidemic is chiefly, if not entirely, due to conditions brought about by the operation of the Lloyd Barrage Scheme.

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TABLE I.

Results of spleen and blood examinations in four schools in Larkana town.

Date.	SPLEEN EXAMINATIONS.				BLOOD EXAMINATIONS.					
	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*	Number examined.	Parasite rate.	M. T.	B. T.	Mixed M. T. and B. T.	Crescents.
SHAH MOHAMED MUNICIPAL SCHOOL.										
1932.										
December ..	300	175	58.3	6.8	70	27.0	16	3	0	3
1933.										
April ..	347	200	57.6	7.4	50	20.0	6	4	0	0
May ..	250	145	58.0	7.3	250	17.8	14	29	1	3
June ..	359	146	40.7	8.3	359	6.4	17	6	0	2
August ..	50	32	64.0	8.8	50	10.0	0	5	0	0
September ..	200	100	50.0	7.9	200	18.5	16	21	0	1
October ..	100	55	55.0	7.0	100	16.0	15	1	0	3
December ..	250	147	58.8	6.0	100	25.0	23	2	0	2
1934.										
January ..	100	56	56.0	8.0	100	11.0	11	0	0	2
March ..	275	155	56.4	7.3	100	10.0	9	1	0	0
TOTAL ..	2,231	1,379
MAIN MUNICIPAL SCHOOL.										
1932.										
December ..	250	131	52.6	7.1	100	20.0	19	1	0	2
1933.										
April ..	200	113	56.5	7.8	200	19.5	22	11	6	4
May ..	283	171	60.4	7.7	283	17.0	19	25	4	7
June ..	300	127	42.3	7.7	200	4.0	8	0	0	2
August ..	150	85	56.7	8.5	150	13.3	12	8	0	0
October ..	100	57	57.0	7.2	100	32.0	17	15	0	2
December ..	160	102	63.7	8.0	100	28.0	22	2	4	7
1934.										
January ..	100	62	62.0	8.0	100	17.0	17	0	0	5
March ..	250	138	55.2	8.0	100	17.0	17	0	0	1
TOTAL ..	1,793	1,333

* Measurement in centimetres from apex of spleen to umbilicus.

TABLE I—concl'd.

Date.	SPLEEN EXAMINATIONS.				BLOOD EXAMINATIONS.					
	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*	Number examined.	Parasite rate.	M. T.	B. T.	Mixed M. T. and B. T.	Crescents.
NAWA TAK SCHOOL.										
1932.										
December ..	53	42	79.2	6.9	30	26.7	7	1	0	0
1933.										
April ..	37	28	75.7	7.6
June ..	50	32	64.0	8.2	50	2.0	1	0	0	0
August ..	50	42	84.0	7.5	50	6.0	3	0	0	0
September ..	50	40	80.0	8.0	50	12.0	4	2	0	0
October ..	50	41	82.0	7.5	50	32.0	16	0	0	9
December ..	55	46	83.6	6.7	50	38.0	16	2	1	1
1934.										
January ..	50	42	84.0	7.0	50	16.0	7	1	0	0
March ..	50	43	86.0	7.2	50	14.0	6	1	0	0
TOTAL ..	445	380
LAHARI SCHOOL.										
1932.										
December ..	50	45	90.0	7.4	45	31.0	14	0	0	1
1933.										
April ..	51	49	96.0	6.8	50	26.0	7	6	0	0
June ..	62	46	74.2	7.4	62	4.8	0	3	0	0
August ..	50	46	92.0	7.6	50	4.0	1	1	0	0
September ..	50	43	86.0	6.6	49	18.4	6	3	0	0
October ..	50	47	94.0	6.4	50	24.0	11	1	0	2
December ..	50	48	96.0	6.6	50	22.0	10	0	1	0
1934.										
January ..	50	47	94.0	6.0	50	12.0	5	1	0	0
March ..	50	46	92.0	6.8	50	12.0	6	0	0	0
TOTAL ..	463	456

* Measurement in centimetres from apex of spleen to umbilicus.

TABLE II.
Combined results of spleen and blood examinations in four schools in
Larkana town, December 1932 to March 1934.

Date.	SPLEEN EXAMINATIONS.				BLOOD EXAMINATIONS.									
	Number ex- amined.	Number with en- larged spleen.	Spleen rate.	Average enlarged spleen.*	Number ex- amined.	Number found infected.	Parasite rate.	Average positive parasite count.†	M. T. parasite rate.	Average positive M. T. count.†	B. T. parasite rate.	Average positive B. T. count.†	Crescent rate.	Average positive crescent count.†
1932.														
December ..	653	393	60.2	7.9	245	61	25.0	1,216	22.9	1,095	2.0	1,256	2.4	1,035
1933.														
April ..	635	390	61.4	7.5	300	62	20.7	561	13.7	676	9.0	395	1.3	35
May ..	533	316	59.3	8.1	533	92	17.2	564	7.1	153	11.1	698	1.9	34
June ..	771	351	45.5	8.4	671	35	5.2	517	3.9	508	1.3	493	0.6	115
August ..	300	205	68.3	8.2	300	30	10.0	1,081	5.3	539	4.7	1,700	0.0	..
September ..	300	183	61.0	7.7	299	52	17.4	911	8.7	660	8.7	646	0.3	120
October ..	300	200	66.7	7.0	300	76	25.3	2,635	19.7	2,462	5.7	2,380	5.3	789
December ..	515	343	67.8	7.9	300	83	27.7	1,400	25.7	1,255	4.0	560	3.3	174
1934.														
January ..	300	207	69.0	7.3	300	42	14.0	545	13.3	445	0.7	70	2.3	63
March ..	625	382	66.3	7.4	300	40	13.3	1,376	12.7	1,446	0.7	30	0.3	120
TOTAL ..	4,932	2,970	3,548	573

* Measurement in centimetres from apex of spleen to umbilicus.

† Per cubic mm. of blood examined.

TABLE III.
Relation of species of parasite and parasite count to size of spleen.

Size of spleen. (Apex-umbilicus measurement in centimetres.)	Number examined.	Number found with para- sites.	M. T. COUNT PER C.M.M. OF BLOOD.				Total found with M. T. parasites.	Percentage found with M. T. parasites.	B. T. COUNT PER C.M.M. OF BLOOD.				Total found with B. T. parasites.	Percentage found with B. T. parasites.	B. T. COUNT PER C.M.M. OF BLOOD.					Total	4
			1-100.	101-500.	501-1,000.	1,001-5,000.			5,001-10,000.	Over 10,000.	1-100.	101-500.			501-1,000.	1,001-5,000.	5,001-10,000.	Over 10,000.			
Not palpable ..	1,375	117	8.5	71	5.1	29	30	6	5	1	0	46	3.3	7	25	10	3	0	1		
14-12 ..	129	23	17.8	19	14.7	0	12	4	2	1	0	4	3.1	1	2	1	0	0	0		
11-8 ..	1,063	209	19.6	151	14.2	39	55	17	27	7	6	58	5.4	14	25	8	9	1	1		
7-4 ..	832	177	21.3	136	16.3	39	51	12	24	4	6	41	5.0	6	19	7	6	1	2		
3-0 ..	125	27	21.6	20	16.0	5	8	.3	3	0	1	7	5.6	3	3	0	1	0	0		
Below umbilicus	7	3	42.9	3	42.9	0	1	0	2	0	0	0	..	0	0	0	0	0	0		
TOTAL ..	3,531	556	15.7	400	11.3	112	157	42	63	13	13	156	4.4	31	74	26	19	2	4		

N.B.—Cases showing mixed infections have been omitted from this Table.

AN EXPERIMENT WITH PARIS GREEN IN A HYPERENDEMIC VILLAGE IN SIND.

BY

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INTRODUCTION.

THE experiment described in the following pages was carried out in the village of Walid, situated in a hyperendemic rice-growing area in Sind, two miles from Larkana, the headquarter town of the district which bears the same name.

The village, which has a population of about 1,200, lies on the bank of the Ghar Canal, one of the old inundation canals of Sind, which originated from the Indus about 30 miles south of Sukkur.

It contains about 100 houses, built of mud and wood. The houses are dark and ill-ventilated, and the streets are tortuous and narrow. Cattle are kept either inside the dwelling-places or in byres adjoining them. Three-fourths of the inhabitants are Mohammedans, and almost all are engaged in the cultivation of the soil. In spite of the very high prevalence of malaria, the general nutrition of both adults and children appears to be satisfactory.

The object of the experiment was to ascertain whether the incidence of malaria in the village could be controlled, or at least mitigated, by conducting an antilarval campaign for a limited period each year. It was realized from the outset that no measure could be of any value as a contribution towards solving the rural malaria problem unless it could be accomplished at a very

low cost. Hence our aim was to limit to the greatest possible extent both the period during which antilarval work was carried out, and the area over which control was attempted.

Antilarval measures were carried out during the years 1930–1933, *i.e.*, during four malaria seasons. Control observations were made in the neighbouring village of Machi during 1930; but as this village is situated so near to Walid that the antilarval work might be held to affect it also, the village of Dodai, which lies $2\frac{1}{2}$ miles to the north of Larkana, was used for control observations during the subsequent three malaria seasons.

Dodai is not on the main Ghar Canal, but has minor distributaries from the canal flowing close to it. These two villages, Walid and Dodai, formed the subject of a previous paper dealing with the factors influencing the normal autumnal malaria incidence in the rice-growing area (Covell and Baily, 1930).

As the result of our previous observations, we had come to the conclusion that the only malaria carrier of any importance in the district was *A. culicifacies*; that the malaria season lasted from September to December, October and November being the most malarious months; and that there was normally an 'infection-free' period, lasting approximately from January to August, except for a short period in February, when infection might possibly take place.

The average annual rainfall, which occurs almost exclusively during the months of July, August and September, is about three inches, but the amount varies considerably from year to year. In 1917, for instance, there were 16 inches of rainfall, and in 1929 ten inches. In each of these years there was a severe regional epidemic of malaria throughout Northern Sind. Owing to the impervious nature of the soil, a comparatively small amount of rainfall causes serious flooding, and the breeding places thus formed remain in existence for long periods. The monthly rainfall figures for the period 1930–1933 are given in Table I.

Temperature and relative humidity figures recorded from August to December during the period 1930–1933 are given in Table II. From January 1931 the readings were taken from instruments fixed in a Stevenson's screen in a garden in Walid. The figures for 1930 were recorded in the verandah of the laboratory in Larkana. It will be observed that there was a considerable rise in the humidity figures in 1932 and 1933, *i.e.*, after the commencement of the working of the Barrage Scheme.

The subsoil water level in the area varies from three to eight feet below ground level during the summer months, when irrigation is in progress, and from five to twelve feet in the non-irrigation season.

Breeding of *A. culicifacies* commences in September, and larvæ of this species are abundant throughout the malaria season. In December and January they are still present, but in diminished numbers. By the end of February active breeding recommences, but during the summer months larvæ of this species are no longer to be found.

An important factor, which influenced both the course and results of the antilarval work, was the operation of the Lloyd Barrage Scheme, which commenced in 1932. When the antilarval work was begun in 1930, it was understood that no new distributaries were to be constructed in the vicinity of Walid,

and that the supply of water in the original channels would not be altered to any great extent; and it was thus thought that the factors influencing the incidence of malaria in the area would not be greatly affected by the operation of the Barrage Scheme. As will be seen, however, conditions were very considerably changed during the latter half of the experiment.

During the period 1930-1931, the principal breeding places of *A. culicifacies* in the sanitated area were:—

(1) The Ghar Canal, flowing from east to west along the southern border of the village. This acted as a prolific breeding place from the beginning of October till the middle of March.

(2) Three distributaries originating from the Ghar Canal, two from the right bank and one from the left. The first two run northwards along the eastern and western borders of the village respectively. The third originates opposite to the village, and carries water to the Government Agricultural Farm. Larvæ of *A. culicifacies* were found in the beds of all three distributaries when the water level in the Ghar Canal fell, towards the end of the irrigation season.

(3) The village pond or tank, 120 yards long and 60 yards broad, which lies about 100 yards from the south-western corner of the village. Larvæ of *A. culicifacies* were found in this, and in the channel supplying it, from September to December.

(4) Seventeen wells, of which 7 were used for domestic purposes, and 10 for irrigation.

(5) Certain borrow-pits and small irrigation channels in the vicinity of the village.

The Lloyd Barrage Scheme provides irrigation for the whole of the rice-growing area on the right bank of the Indus by means of the new Central Rice Canal, which originates, like the other new great canals, immediately above the Barrage at Sukkur. This cuts across the Ghar Canal about half a mile to the south-east of Walid, and supplies water to it from a new head, provided with a regulator. As a result, a portion of the old canal, between Walid and the Central Rice Canal, was in 1932 converted into a pond, which became a breeding place for *A. culicifacies*.

Secondly, during the year 1932, the Ghar Canal did not receive an adequate supply of water. Consequently, the distributaries arising from it were only partially filled, and mosquito breeding in them was thereby increased.

Thirdly, about 4 feet of water was allowed to remain in the bed of the Central Rice Canal after the end of the irrigation season, in order to strengthen its banks, thus providing a favourable breeding place for *A. culicifacies*.

Fourthly, during the irrigation season the upper portion of the old Ghar Canal, now cut off by the Central Rice Canal, situated immediately north of Larkana town, became a prolific breeding ground for *A. culicifacies*. After the irrigation season, this portion of the canal was flushed at frequent intervals into the Central Rice Canal, and from thence into the lower portion of the Ghar Canal. In this way, large numbers of larvæ were carried into the portion of the canal running past Walid village.

In the year 1933, in consequence of the failure in the previous year to supply sufficient water for the lands surrounding Walid village, two new canal distributaries were excavated. One of these, the Walid Minor, originates from the Central Rice Canal, and runs within 100 yards of the northern border of

the village. The other, the Hume Pipe Channel, runs parallel with the Ghar Canal, on the opposite side from the village. These two distributaries, together with the small channels coming off from them, and the borrow-pits along their course, afforded additional breeding places for *A. culicifacies*. Further, in order to drain off the excess of water from the fields, the level of the water in the Central Rice Canal was periodically lowered during the irrigation season for a week or more at a time. Larvæ breeding in the stagnant water of the canal were subsequently washed into the Ghar Canal and the two distributaries mentioned above. As in the previous year, the upper portion of the Ghar Canal was periodically flushed, so that larvæ were carried from it into the sanitated area. At one period the Ghar Canal was treated daily with paris green for about 20 days, but half-grown larvæ were found within 24 hours of dusting, as before.

Thus it will be seen that during the years 1932 and 1933 the problem of larval control round Walid village became highly complicated, breeding conditions being very different from those obtaining in the first two years of work. To further complicate matters, there was an unusually heavy rainfall in 1933 (11 inches), 9 inches falling in August, and more than one inch in July.

ANTILARVAL OPERATIONS.

The only antilarval measure employed was the dusting of breeding places with paris green for a distance of 500 yards from the periphery of the village in each direction. The sanitated area was divided into five portions for this purpose, namely (1) north, (2) south, (3) east, (4) west, and (5) the village proper.

The amount of paris green used was calculated at approximately 1 lb. per acre of water surface treated. The mixture was applied partly by means of rotary blowers, and partly by hand throwing. The diluent was powdered soapstone or slaked lime when blowers were used, and road dust when the mixture was applied by hand. Various dilutions were tried, from 1 to 5 per cent by volume when blowers were used, and from 1 to 2½ per cent for hand dusting. The most effective dilutions were found to be 2½ per cent by volume for blowers, and 1 per cent for hand throwing. The intervals between dusting were 5 to 7 days from August to the end of October, and from 7 to 9 days from 1st November onwards.

The staff employed consisted of one sub-assistant surgeon supervising the work, one insect collector and one coolie. The coolie was employed only for the actual period of antilarval operations each year, i.e., from 1st August to 31st December, and it was not possible to obtain the services of the same man each year. The sub-assistant surgeon and insect collector were working for the remainder of the year on other duties connected with the Sind Malaria Inquiry.

The cost of the work during the four years under review is shown in Table III. The wages of the coolie are included, but not those of the sub-assistant surgeon and insect collector. It will be observed that the cost of operations was very considerably increased during the last two years, owing to the necessity of dealing with the altered breeding conditions described above. The cost per head of population works out at roughly 2·2, 3, 4·3 and 4·8 annas for each of the four years respectively, but it should be realized that conditions

in 1932 and 1933 were altogether abnormal. The initial expenditure on apparatus, which is not included above, came to Rs. 306-3-0. This includes the cost of two blowers. One of these would have been sufficient for the work, and the price of the blowers has since been decreased, so that the initial cost of apparatus at the present time may be estimated at about Rs. 140.

The effect of the work was tested by (1) search for larvæ in breeding places, (2) search for adult mosquitoes in catching stations, (3) dissection of mosquitoes, and (4) periodical spleen and blood examinations of the village children. These observations were made both in Walid and in the unsanitated village of Dodai.

The catching stations each consisted of two houses, situated in the north, south, east, west and central portions of each village. Thus there were 10 houses to be searched in each village. A period of 10 minutes was given for searching each house. All the mosquitoes caught during this period were taken back to the laboratory for identification and dissection. Whenever there was an increase in the number of mosquitoes collected in any of the catching stations in Walid, the cause was sought for, and steps taken to deal with it.

RESULTS OF OBSERVATIONS.

(a) COLLECTION OF ADULT MOSQUITOES.

The numbers of specimens of *A. culicifacies* collected in the catching stations in Walid and Dodai during the period 1930-1933 are shown in Tables IV and V. It will be seen that the average numbers collected daily in Walid in 1930 and 1931 were 7 and 5 respectively. Collecting was commenced in Dodai in 1931, when the average daily catch was 27, i.e., more than 5 times as many as were caught in Walid during that year. In 1932 the average catch in Walid rose to 11, as against 39 in Dodai, whilst in 1933 the respective numbers were 9 and 42. In Walid, in each year the largest numbers were caught in the southern station, which was close to the Ghar Canal. The marked increase in numbers in 1932 and 1933 was however noticeable in the eastern and western, as well as in the southern stations.

(b) INFECTIVE DENSITY OF ANOPHELINES.

Davey and Gordon (1933) have proposed a formula for calculating the density of infective anophelines, which, as they pointed out, is the main factor influencing the inoculation rate. The formula is as follows:—

$$\frac{\text{Total number of female anophelines captured} + 'x'}{\text{Total number of rooms examined}} \times \frac{\text{Total number of sporozoite-infected anophelines}}{\text{Total number of female anophelines dissected.}}$$

'X' represents the proportion of mosquitoes leaving the houses before dawn, and must be taken into account in places where it is known that the proportion leaving before dawn is significantly large. But, when comparing together infective densities of different localities, so long as the anophelines behave similarly as regards the period they remain in the house after their blood meal, the modifying term 'x' does not require to be taken into consideration, as it is a factor common to both the infective densities. In the present instance, since *A. culicifacies* is the only anopheline to be considered, 'x' can be ignored.

Davey and Gordon found that in Freetown (West Africa), where control measures were practised, there was an anopheline infective density of 0.024, whilst in Ibadan, Lagos and Kissy the corresponding figures were 0.098, 0.403 and 0.844 respectively. From this they calculated that the anopheline infective density in Kissy was 34 times as great as in Freetown, and that in Ibadan it was 4 times, and in Lagos 17 times as great. They point out that, whereas the individual risk of inoculation with malaria cannot as yet be estimated accurately from the anopheline infective density, yet the former depends on the latter, for they proved that a close correlation exists between a high infective density and a high malaria rate amongst children examined during the first three months of their life, while a low infective density is associated with a low infection rate.

The infective densities of *A. culicifacies* in the villages of Walid and Dodai during the period 1930-1933 are shown in Table VIII. In Walid the figure was 0.012 in 1930, but fell to 0.003 in 1931. In 1932 it rose to 0.016, and the same figure was recorded in 1933. When these figures are compared with those for Dodai, it is seen that the infective density of *A. culicifacies* in 1931 was 13 times as high in the latter village. In 1932 it was rather more than three times as high, and in 1933 rather less. Details of both salivary gland and mid-gut dissections carried out in the two villages are given in Tables VI and VII.

(c) SPLEEN EXAMINATIONS.

The results of spleen examinations among the children of the two villages are given in Table IX. The first observations, in November 1927, yielded spleen rates of 61 per cent in Walid and 54 per cent in Dodai. In July 1928, the respective figures were 37 and 53. In November 1929, at the height of the severe regional epidemic which occurred during that year, there was a spleen rate of 91 per cent in Walid, and 88 per cent in Dodai. The average enlarged spleen (A.E.S.) was 6.3 in Walid and 6.4 in Dodai (apex-umbilicus measurement in cm.). Thus, in the year preceding the commencement of antilarval operations, the conditions as revealed by spleen examination were about equal in the two villages. In July 1930, the respective spleen rates were 55 per cent and 66 per cent, and the A.E.S. were 6.9 and 6.7 cm.

During the malaria season of 1930, the first year of antilarval work, the spleen rates rose to 91 per cent in Walid and 94 per cent in Dodai, the A.E.S. being 7.4 and 6.9 respectively. In 1931 there was a slight reduction in the spleen rate in Walid, to 84 per cent as against 95 per cent in Dodai, and a considerable reduction in the size of the A.E.S., which was about 3 cm. larger in Dodai than in Walid.* In 1932 there was a marked reduction in the spleen rate in Walid, to 40 per cent in July and 56 per cent in August. The rate in Dodai in August was 82 per cent. There was again a considerable difference in the A.E.S. of the two villages. In the autumn of 1932, however, the spleen rate in Walid again rose, reaching 88 per cent in November, as against 98 per cent in Dodai. During the dry season of 1933, the lowest rates recorded were 65 per cent in Walid and 77 per cent in Dodai (May observations). In the autumn of 1933, the spleen rates in both villages were again very high, reaching 94 per cent in each case in December. There was still, however, a considerable

* It must be remembered that the apex-umbilicus measurement of the average enlarged spleen decreases as the spleens increase in size.

difference in the A.E.S. (6.9 cm. in Walid, and 5.8 cm. in Dodai). In August 1934, a number of children born since the commencement of antilarval measures were examined. The spleen rate amongst these children in Walid was 56 per cent (45 observations), whilst in Dodai it was 79 per cent (24 observations).

(d) BLOOD EXAMINATIONS.

The results of blood examinations made periodically in the two villages are given in Tables X and XI. In July 1928 the parasite rates were approximately the same in both villages (21 per cent). During the epidemic of 1929 the rates in both cases were very high (76 per cent in Walid and 74 per cent in Dodai, in November). The only comparable observations in 1930 were made in July and September, when the rates were 23 and 74 in Walid, and 26 and 47 in Dodai. During the malaria season of 1931, rates of 59, 80 and 64 were recorded in Walid, as against 28, 36 and 36 in Dodai. Only 25 children were examined in each village on each visit, but even so it is noteworthy that the rates in Walid were about double those in Dodai. In the autumn of 1932, Walid yielded rates of 21, 36 and 36, whilst in Dodai at the same period the figures were 36, 52 and 34. During the malaria season of 1933, the figures at Walid were 23, 23 and 26, whilst at Dodai they were 6, 16 and 2 only.

DISCUSSION OF RESULTS.

There are many pitfalls to be encountered in estimating the value of anti-malarial measures. Conditions rarely remain static from year to year, and it is only by comparing observations made in the area under control with those made in an unsanitated locality where conditions are similar, that a just evaluation of results can be attained.

In the present instance it has been shown that conditions did not remain stable during the four years of antilarval work. Breeding conditions of anophelines were profoundly modified by the operation of the Lloyd Barrage Scheme during the years 1932 and 1933. There is also some evidence that the operation of the Scheme has resulted in a prolongation of the period favourable to malaria transmission, by increasing the relative humidity of the atmosphere. Other factors to be taken into account are the regional epidemic of malaria of 1929, and the unusually high rainfall of 1933.

An analysis of the results of observations carried out in the two villages shows that, while the antilarval measures carried out during the first two years had a considerable effect, they failed to deal adequately with the adverse conditions obtaining in 1932 and 1933. The publication of results which have failed to achieve their object is perhaps unusual. Frequently, however, more can be learnt from failures than from successes, and it is felt that a critical examination of our results may be of value to other workers engaged on the problem of malaria control in rural areas.

The results of mosquito collections in the catching stations show that in 1930 and 1931 the reduction in the numbers of *A. culicifacies* in Walid was very considerable. It is unfortunate that the observations were not carried out for a season before antilarval work was started, and also that figures are not available for the control village for 1930. However, the daily average catch of 5 in Walid in 1931, compared with 27 in Dodai, is an indication that the

number of *A. culicifacies* in the former village was probably cut down by something like 80 per cent in that year. The rise of the average catch to 11 in 1932 and 9 in 1933 indicates a partial failure in the antilarval work. Even so, the numbers were only one-third and one-fourth respectively of the corresponding average catch in the control village.

It has been noted above that the infective density of *A. culicifacies* was 13 times as high in Dodai as in Walid in 1931, but only about 3 times as high in 1932 and 1933. This again points to a considerable success in the antilarval work during the first two years of the experiment, followed by less effective work in 1932 and 1933.

The results of spleen examinations were interesting. As has been noted, the spleen rates and the size of the average enlarged spleen in the two villages were almost exactly equal immediately before antilarval work was started. In the malaria season of 1930 the spleen rates in both villages rose to over 90 per cent. In 1931 the A.E.S. in Walid was much smaller than in Dodai, though the spleen rate was only slightly reduced. Probably in each village every person received at least one infection, but the larger A.E.S. recorded in Dodai suggests that the number of infections and re-infections in that village was the greater. In other words if Walid represented a condition of saturation with malarial infection, Dodai was super-saturated. This view is supported by the figures recorded in the following August, when the spleen rate had dropped to 55 per cent in Walid, whilst it was 82 per cent in Dodai. The high spleen rates recorded in Walid in the malaria seasons of 1932 and 1933 confirm the conclusions reached from the results of anopheline catches that the antilarval measures in those years failed to maintain their initial success.

As regards the blood examinations, the parasite rates recorded during the three malaria seasons when comparable figures are available were higher in Walid than in Dodai in 1931 and 1933, and slightly lower in 1932.

To summarize, the evidence indicates that the antilarval measures met with some success in reducing the incidence of malaria in Walid during the first two years; but that they failed to maintain this success owing to the changed conditions produced by the operations of the Lloyd Barrage Scheme.

THE PROBLEM OF RURAL MALARIA CONTROL IN TROPICAL COUNTRIES.

The subject of malaria control in the tropics may conveniently be grouped under three headings, (1) the control of *urban* malaria, (2) the control of *industrial* malaria (by which we mean malaria among labour forces on estates or plantations, railway communities, etc.), and (3) the control of *rural* malaria.

The experience gained in many parts of the world during the last 30 years has shown us that malaria can probably be controlled anywhere, *provided that sufficient funds are available*. There are many instances in tropical countries of successful control in the case of urban and industrial malaria. In both these instances, it can usually be shown clearly that the cost of malaria to the community is far greater than the cost of effective control.

It is the control of rural malaria which presents the great problem to the malariologist in tropical countries. It is true that malaria has been controlled in rural areas in certain instances, but the cost of the measures employed has

usually been far greater than the authorities concerned can afford. As to the choice of control methods, it is generally conceded that up till now mass treatment of the population alone has had but little effect; antimosquito measures have been found successful, but at too great a cost; whilst in some instances these two methods have been combined with satisfactory results, but again at too great a cost. The difficulties and complexity of the problem have frequently been stressed in the literature relating to malaria control in the tropics.

Ross (1911) was careful to emphasize that he did not advocate antimosquito measures in all circumstances. 'This proposal was obviously not meant to apply to the whole world, but only to places where the measures were likely to be most feasible, that is, generally to "crowded areas", and not to "rural areas"'. A square mile of town, containing thousands of rate-payers, may be assumed to have money available for such work; while open country, containing perhaps only a few scattered houses, has no such funds. . . . It goes without saying that we can scarcely ever attempt to deal with *Anopheles* in large rural areas'.

Sweet and Rao (1934) lay special emphasis on the vital necessity of adequate supervision. 'Successful control work cannot be carried out without an adequate technically trained staff on full-time duty. In other words, control work under the supervision solely of the general health officer or sanitary inspector will not be a success, but will usually be a waste of any money that may be spent upon it. . . . Anopheline control and tests of its results are of too technical a nature, and require too much expert supervision, to allow of their being placed under the necessarily casual control of officers who have other duties to perform'.

Scharff (1929), speaking of antimalaria work in Malaya, remarks 'Money is as hard to obtain for health work in Malaya as anywhere else in the world, and it is only because antimosquito work is proved to be a paying proposition that the Government and the public alike support it'.

Barber and Olinger (1931), referring to their work in West Africa, state 'Antilarval measures meet with special difficulties. Larvæ are adapted to a great variety of breeding places, and their development is very rapid, enabling them to take advantage of very temporary collections of water. Adults are domestic in their habits, very susceptible to malarial infection, and capable of a wide dispersion. For many years to come, any extensive antilarval measures will probably have to be confined to the protection of towns or other limited areas. . . . We doubt if any larvicidal campaign can alone cope with conditions in West Africa'.

Hackett (1929) says, 'I think it is justly held that organized larva control is not economical in most thinly scattered rural populations. Here, the burden must be shifted from the community to the individual'.

Kligler (1926) notes that to obtain successful results by mass treatment in Palestine would be more difficult and more costly than antilarval operations. 'With good mosquito control, there is a steady reduction of the spleen rate, whether or not it is accompanied by treatment'. The cost of the antilarval measures referred to, however, would be out of the question in rural India (*vide infra*).

Russell (1933) gives a vivid account of the difficulties of antimalaria work in rural areas in the Philippines. He points out that there is no example of effective control in the tropics by measures which did not include an attack on anopheline mosquitoes. 'Given plenty of money, effective malaria control can be accomplished in a relatively short time. In Panama practical control was quickly realized, but . . . malaria has never been entirely eradicated from the Canal Zone'. He goes on to enumerate the difficulties encountered amongst a population who will not endure the discomfort of nets or screening, or of medication, and who are ignorant of hygiene and nonchalant towards illness and death. Labour is undependable, and pervading all the factors which militate against effective malaria control in the tropics is the paralysing lack of funds. He does not despair, however, of ultimate success. 'Antilarval measures, plus bed-nets and drugs, should bring about results *in time*, provided a modest educational campaign and continued research is coincident with them'.

As regards the costs of antilarval measures in various countries, the following figures given by various workers are of interest:—

Gorgas (1911) states that the total cost of sanitation in the Panama Canal Zone was 3½ dollars per head per annum, of which 2 dollars was spent on mosquito work.

In the annual report of the Public Health Commissioner with the Government of India for 1932 it is stated that 'the total sum allowed for antimalaria field work for the army in India for the year represented an expenditure per head of the military population of 10*d.*, whereas in the last year for which figures are available, the expenditure on such field work in the Panama Canal Zone amounted to almost £1 16*s.* per head'.

Sweet and Rao (1934) estimate the cost of malaria control in Mysore State as from Rs. 2 to Rs. 6 per head per annum in villages of 500 to 2,000 population, and from As. 12 to Re. 1-5 in villages of 2,000 to 5,000 population. But they add that it might be possible to carry out efficient control in a compact area containing a number of large villages for from As. 8 to Re. 1-5 per head, with a staff of one field labourer per village (with extra men for replacement, mixing of paris green, etc.), one technical inspector for 10 villages, and one malaria officer for a maximum of about 60 villages.

Scharff (1929) states that the cost of antimalaria work in rural Singapore is 1.65 dollars per head (3*s.* 8*d.*), capital cost, and 45 cents (1*s.*) per head per annum for maintenance of existing works and oiling. The area is divided into five sanitary districts, each of 60 square miles, with a fully trained sanitary inspector for each district. The central supervising and laboratory staff consists of one chief sanitary inspector, a qualified drainage inspector and two surveyors, a laboratory assistant, and three mosquito collectors.

Hackett (1929) found that the cost of antilarval work in Portotorres, Sardinia, was about 7*d.* per head per annum, whilst in Bianconovo, Southern Italy, the cost was only about one-third of this per head of population. Each of these rural towns had a population of about 6,000. In both these instances, however, the towns were situated on the sea, so that the area to be controlled was only half as large as for an inland town. Moreover, the malaria season

occurs at a time when there is no rainfall, so that breeding places remain unaltered throughout the period of control.

Kligler (1926) found that effective mosquito control in Palestine in a new district amounted to 1.50 dollars per head, but that in subsequent years the cost was reduced to 0.50 dollar.

Russell (1935) states that the practical limit of expenditure for routine malaria control work possible for the average town of 10,000 inhabitants in the Philippines, over and above salaries and expenses of public health personnel already existent, is not more than 5 cents per head per annum ($2\frac{1}{2}d.$), and it would be impossible to obtain as much as this in many places. He states further that, whilst there have been successes in antilarval control in the Philippines, the cost has been five or six times as great as this.

Le Prince (1931) computes the cost of antimalaria control in the U. S. A. at 0.70 to 0.80 dollar per head in the first year, and 0.2 to 0.3 dollar in subsequent years.

The figures given above represent an expenditure far greater than could be afforded by the average Indian village. Indeed, it is doubtful whether in most cases any money at all would be available for antimalaria work.

On the whole, it cannot be said that the opinions which have been cited with regard to the problem of malaria control in rural areas in the tropics are encouraging. Our experience in Indian villages has in the main confirmed the conclusions arrived at by other workers as to the difficulties encountered. The remarks of Barber and Olinger and of Russell regarding the problem of malaria control in West Africa and in the Philippines in particular are equally applicable in India.

We feel that the only prospect of any success lies in the establishment of malaria units in rural towns which are of sufficient size to warrant the expenditure of the necessary amount of money. The work might then be gradually extended to a circle of neighbouring villages, provided that there was passably good communication by road between these and the central town. In this way the cost of skilled supervision, which is so essential for success, whatever anti-malarial measure be adopted, would be spread over a number of villages.

As regards education and propaganda, this should be directed principally in the direction of medical treatment and the popularizing of the use of bed-nets. The process of inculcating the first principles of rural hygiene must necessarily be slow, but this should not be made a reason for abandoning our efforts.

SUMMARY AND CONCLUSIONS.

1. An account is given of an experimental attempt to control malaria in a hyperendemic village in Sind, by means of paris green dusting of anopheline breeding places carried out during a limited period each year.

2. Partial success was achieved during the first two years of operations, but this was not maintained during the subsequent two years. It is considered that this was due to the altered breeding conditions brought about by the operation of the Lloyd Barrage Scheme.

3. Antilarval measures in rural areas cannot succeed without constant and careful skilled supervision. In the present instance, in spite of supervision

by a trained sub-assistant surgeon, the dusting of breeding places with paris green for a distance of 500 yards from the periphery of the village failed to control malaria under the altered conditions produced during 1932 and 1933.

4. Paris green is not a suitable larvicide for dealing with moving water, at any rate under the conditions of canal irrigation. The use of oil balls tethered along the margins of the canal would probably have been much more effective in killing the larvæ which were constantly being washed into the sanitated area.

5. Antilarval measures are not likely to meet with any dramatic success under hyperendemic rural conditions in Sind, because the anopheline density necessary to maintain a high rate of malarial infection is so low. In the present instance, even after a reduction of 80 per cent in the number of malaria-carrying mosquitoes in Walid, the number of infections was sufficient to produce a spleen rate of over 80 per cent in the autumn of 1931.

6. Lack of funds presents the greatest obstacle to the control of malaria in rural areas. To attain the partial success achieved during the first two seasons our antilarval work cost between two and three annas per head of the population per annum. It is considered that under normal circumstances this would represent the minimum recurring charges for materials and labour for effective antilarval work in villages in Sind. But this does not take into account the cost of the continuous skilled supervision which is so vitally necessary, nor the initial expenditure on apparatus.

7. The problem of the control of rural malaria in tropical countries generally is discussed. The difficulties described by other workers are in the main confirmed. In the authors' opinion the control of malaria in hyperendemic rural areas in India is not likely to be achieved by antilarval measures under the present conditions.

8. We consider that the only practical possibility of carrying out effective antilarval work in Sind would be to establish antimalarial units in headquarter towns, and gradually extend the work into the villages in the immediate neighbourhood, so that the cost of skilled supervision could be spread over a number of villages. This, however, could only be successfully carried out where a number of large villages exist within a short distance of the headquarter town, connected with it by passably good roads. Except under these exceptionally favourable conditions, we do not consider that the prosecution of effective antilarval measures in villages in Sind is a practical proposition.

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TABLE I.

Monthly rainfall in inches recorded in Larkana, 1930-1933.

Months.	1930.	1931.	1932.	1933.
January	0·07
February	0·31	..	0·40
March	0·10	0·22
April	0·30	..	0·12
May	0·46	0·04
June	0·53
July	1·15	..	2·74
August	0·70	..
September	0·03
October
November
December
TOTAL ..	1·98	1·01	3·67	11·45

TABLE II.
Temperature and relative humidity figures recorded in the months August to December during the years 1930-1933.*

Months.	1930.						1931.						1932.						1933.					
	Mean temperature, °F.	Mean relative humidity, per cent.	Average temperature, °F.		Mean temperature, °F.	Mean relative humidity, per cent.	Average temperature, °F.		Mean temperature, °F.	Mean relative humidity, per cent.	Average temperature, °F.		Mean temperature, °F.	Mean relative humidity, per cent.	Average temperature, °F.		Mean temperature, °F.	Mean relative humidity, per cent.	Average temperature, °F.					
			maximum	minimum			maximum	minimum			maximum	minimum			maximum	minimum			maximum	minimum				
August ..	90.2	73.3	93.2	85.2	92.5	70.9	103.1 *	82.0	88.9	77.8	97.5	82.4	88.7	80.0	96.7	80.7	82.4	88.7	80.0	96.7				
September ..	88.1	76.3	92.2	84.0	88.3	65.9	99.1	77.5	88.7	83.3	101.9	75.6	87.0	80.2	96.5	77.5	75.6	87.0	80.2	96.5				
October ..	91.8	66.0	86.0	76.3	80.0	59.1	95.8	64.3	78.6	71.5	96.5	60.8	79.1	74.6	93.7	64.5	60.8	79.1	74.6	93.7				
November ..	72.7	68.5	78.1	67.3	68.8	52.9	86.8	50.9	69.3	80.1	89.6	49.1	69.7	77.8	85.6	53.8	49.1	69.7	77.8	85.6				
December ..	65.9	69.6	71.6	60.3	60.0	48.8	76.8	43.3	59.6	71.2	76.1	43.1	61.8	89.8	77.5	46.2	43.1	61.8	89.8	77.5				

* Figures for 1931, 1932 and 1933 were recorded in a garden in Walid. Those for 1930 were recorded in the verandah of a house in Larkana.

TABLE III.
Yearly expenditure on antilarval measures in Walid, 1930-1933 (excluding salaries of sub-assistant surgeon and insect collector).

PARIS GREEN.	LIME.		ROAD DUST.		SOAPSTONE.		Coolie wages.		Repair charges.		Total cost.		Cost per head of population.	
	Amount used.		Cost.		Amount used.		Cost.		Rail charges.		Rs. As.		Rs. As.	
	lbs.	Rs. As.	Mds.	Rs. As.	Rs. As.	Mds.	Rs. As.	Rs. As.	Rs. As.	Rs. As.	Rs. As.	Rs. As.	Rs. As.	Rs. As.
1930 ..	56	51 10	10½	8 0	2 4	72 4	4 10	138 12	0 2.2
1931 ..	40	35 0	2 8	2	3 0	6 0	56 0	3 8	186 0	0 2.9
1932 ..	48	42 0	1 0	13	19 8	39 0	64 0	4 0	269 8	0 4.3
1933 ..	112	98 0	25	18 12	1 0	80 0	5 0	302 12	0 4.8

N.B.—Slaked lime purchased locally was found as effective a diluent as soapstone. One maund is equal to 82 pounds. The value of one rupee is approximately one shilling and six pence. There are sixteen annas in each rupee.

TABLE IV.

Total monthly and average daily collections of A. culicifacies from August to December in the catching stations in Walid, 1930-1933.

Months.	1930.		1931.		1932.		1933.	
	Total monthly catch.	Average daily catch.	Total monthly catch.	Average daily catch.	Total monthly catch.	Average daily catch.	Total monthly catch.	Average daily catch.
August ..	68	4	10	2	182	11	129	7
September ..	159	8	73	4	219	8	214	11
October ..	91	5	43	5	152	6	183	9
November ..	105	5	129	9	231	9	316	13
December ..	229	13	134	7	152	7	69	4
TOTAL ..	652	7	389	5	936	11	911	9

TABLE V.

Total monthly and average daily collections of A. culicifacies from August to December in the catching stations in Dodai, 1931-1933.

Months.	1931.		1932.		1933.	
	Total monthly catch.	Average daily catch.	Total monthly catch.	Average daily catch.	Total monthly catch.	Average daily catch.
August ..	19	2	608	43	407	27
September ..	236	11	678	36	430	27
October ..	436	36	797	50	647	34
November ..	423	47	689	41	1,215	64
December ..	433	72	432	27	877	52
TOTAL ..	1,547	27	3,204	39	3,576	42

TABLE VI.

Results of dissections of *A. culicifacies* caught in Waid during the period August to December, 1930-1933.

Months.	1930.						1931.						1932.						1933.					
	TOTAL.		MID-GUT.		SALIVARY GLAND.		TOTAL.		MID-GUT.		SALIVARY GLAND.		TOTAL.		MID-GUT.		SALIVARY GLAND.		TOTAL.		MID-GUT.		SALIVARY GLAND.	
	Number dissected.	Number infected.	Number dissected.	Number infected.	Number dissected.	Number infected.	Number dissected.	Number infected.	Number dissected.	Number infected.	Number dissected.	Number infected.	Number dissected.	Number infected.	Number dissected.	Number infected.	Number dissected.	Number infected.	Number dissected.	Number infected.	Number dissected.	Number infected.	Number dissected.	Number infected.
August ..	18	4	47	4	46	0	10	0	9	0	10	0	32	0	31	0	32	0	84	1	81	1	84	0
September	154	18	153	16	154	3	35	0	35	0	35	0	136	1	130	0	132	1	137	2	133	1	135	1
October ..	68	3	68	2	68	1	26	1	25	1	26	0	102	2	85	0	101	2	110	6	107	1	106	5
November	67	11	65	9	67	5	82	1	74	0	81	1	196	11	186	4	195	9	147	6	141	1	141	5
December	164	23	154	22	164	1	80	1	63	0	80	1	140	1	126	0	136	1	51	1	49	1	50	0
Total ..	501	59	487	53	499	10	233	3	206	1	232	2	606	15	558	4	596	13	529	16	511	5	516	11
Percentage infected.	11.6		10.9		2.0		1.2		0.5		0.9		2.5		0.7		2.2		3.0		1.0		2.1	

TABLE VII.

Results of dissections of *A. culicifacies* caught in Dodai during the period August to December, 1931-1933.

Months.	1931.						1932.						1933.					
	TOTAL.		MID-GUT.		SALIVARY GLAND.		TOTAL.		MID-GUT.		SALIVARY GLAND.		TOTAL.		MID-GUT.		SALIVARY GLAND.	
	Number dissected.	Number infected.	Number dissected.	Number infected.	Number dissected.	Number infected.	Number dissected.	Number infected.	Number dissected.	Number infected.	Number dissected.	Number infected.	Number dissected.	Number infected.	Number dissected.	Number infected.	Number dissected.	Number infected.
August ..	13	0	12	0	12	0	85	0	84	0	83	0	209	3	197	3	199	1
September	97	8	93	8	96	1	372	5	356	4	359	2	246	7	236	6	237	1
October ..	285	13	269	11	274	3	408	21	390	13	394	11	313	17	303	8	302	10
November	299	13	270	7	285	6	434	11	421	4	431	8	449	7	425	2	426	5
December	216	10	168	6	213	4	216	1	198	1	208	0	304	8	298	6	299	2
TOTAL ..	910	44	812	32	880	14	1,515	38	1,449	22	1,475	21	1,521	42	1,459	25	1,463	19
Percentage infected.	4.8		3.9		1.6		2.5		1.5		1.4		2.7		1.7		1.3	

TABLE VIII.

Sporozoite rate and infective density of A. culicifacies in Walid and Dodai during the period August to December, 1930-1933.

	WALID.						DODAI.							
	Houses examined.	Females caught.	Rate per house.	Number dissected.	Number with sporozoites.	Sporozoite rate.	Infective density.	Houses examined.	Females caught.	Rate per house.	Number dissected.	Number with sporozoites.	Sporozoite rate.	Infective density.
1930.														
August ..	240	67	0.28	46	0	1.95	0.012
September ..	240	154	0.63	154	3	1.47	0.007
October ..	180	81	0.45	68	1	7.46	0.037
November ..	190	95	0.50	67	5	0.61	0.008
December ..	170	214	1.26	164	1		
TOTAL ..	1,020	611	0.60	499	10	2.00	0.012
1931.														
August ..	170	10	0.06	10	0	80	16	0.20	12	0	1.04	0.009
September ..	230	49	0.21	35	0	220	190	0.86	96	1	1.09	0.034
October ..	100	27	0.27	26	0	120	370	3.08	274	3	2.15	0.090
November ..	150	89	0.59	81	1	1.23	0.007	90	367	4.07	285	6	1.83	0.126
December ..	200	114	0.57	80	1	1.25	0.007	60	404	6.73	213	4		
TOTAL ..	850	239	0.30	232	2	0.86	0.003	570	1,347	2.36	880	14	1.59	0.037

1932.	160	154	0.96	32	0	..	140	564	4.03	83	0	..56	..
August ..	260	193	0.74	132	1	0.76	190	626	3.29	359	2	0.56	0.019
September ..	260	122	0.47	101	2	1.98	160	705	4.41	394	11	2.79	0.123
October ..	260	220	0.85	195	9	4.61	170	624	3.67	431	8	1.86	0.068
November	230	151	0.66	136	1	0.74	160	398	2.47	208	0
December													
TOTAL ..	1,170	840	0.71	596	13	2.18	820	2,917	3.56	1,475	21	1.42	0.050
1933.	210	117	0.56	84	0	..	150	384	2.56	199	1	0.50	0.013
August ..	190	182	0.43	135	1	0.74	160	380	2.37	237	1	0.42	0.010
September	210	146	0.70	106	5	4.71	190	452	2.40	302	10	3.31	0.071
October ..	250	282	1.28	141	5	3.54	190	887	4.66	426	5	1.20	0.055
November	200	67	0.33	50	0	..	170	809	4.76	299	2	0.66	0.031
December													
TOTAL ..	1,060	794	0.75	516	11	2.13	860	2,912	3.40	1,463	19	1.30	0.044

TABLE IX.

Results of spleen examinations among the children of Walid and Dodai during the period 1927-1933.

Date.	WALID.				DODAI.			
	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*	Number examined.	Number with enlarged spleen.	Spleen rate.	Average enlarged spleen.*
xi. 27 ..	100	61	61.0	7.6	33	18	54.5	8.4
vii. 28 ..	80	30	37.5	7.6	43	23	53.5	7.2
xi. 29 ..	93	85	91.4	6.3	76	67	88.0	6.4
vii. 30 ..	90	50	55.0	6.9	50	33	66.0	6.7
viii. 30 ..	118	96	81.3
ix. 30 ..	397	360	90.7	7.4	47	44	94.0	6.9
x. 30 ..	400	385	96.3	7.9
xi. 30 ..	155	147	96.1	6.9
iii. 31 ..	130	123	94.6	7.2
viii. 31 ..	242	200	82.6	8.0	47	43	91.5	6.6
ix. 31 ..	243	205	84.4	8.9	46	42	91.3	7.6
x. 31 ..	203	169	83.2	8.5	43	41	95.4	6.6
xi. 31 ..	228	193	84.6	9.0	58	57	98.2	6.0
i. 32 ..	225	189	84.0	8.8	63	59	93.7	6.4
ii. 32 ..	249	201	80.7	8.9
iii. 32 ..	238	199	83.6	8.9	54	49	91.0	6.9
vi. 32 ..	189	75	39.7	8.2
viii. 32 ..	232	129	55.6	9.3	125	103	82.4	7.3
ix. 32 ..	288	165	57.6	8.8	162	133	82.1	7.0
x. 32 ..	200	135	67.5	8.8	70	64	91.4	6.5
xi. 32 ..	214	188	87.8	7.8	115	113	98.3	5.7
xij. 32 ..	203	172	84.7	7.8	90	89	98.9	5.8
i. 33 ..	204	177	86.7	8.2	92	91	98.9	5.7
ii. 33 ..	190	156	82.1	8.4	76	71	93.4	6.4
iii. 33 ..	213	179	84.0	8.1	75	66	88.0	6.4
iv. 33 ..	183	147	80.3	8.2	76	68	89.5	6.4
v. 33 ..	222	145	65.2	7.8	94	74	77.0	6.2
vi. 33 ..	79	60	76.0	8.0
viii. 33 ..	285	220	77.2	8.1	85	78	91.7	7.5
ix. 33 ..	190	143	75.3	8.3	90	83	92.2	7.4
x. 33 ..	207	172	83.0	7.8	100	89	89.0	6.8
xi. 33 ..	200	186	93.0	6.4	100	94	94.0	6.4
xii. 33 ..	183	173	94.5	6.9	85	80	94.0	5.8

* Measurement in centimetres from apex of spleen to umbilicus.

TABLE X.

Results of blood examinations among the children of Walid, 1927-1933.

Date.	Number examined.	Number found with parasites.	Parasite rate.	Average parasite count per c.mm. of blood.	NUMBERS FOUND WITH				
					M. T.	B. T.	Q.	Mixed B. T. and M. T.	Crescents.
xi. 27 ..	75	35	46.6	1,941	25	8	2	..	13
vii. 28 ..	80	17	21.3	426	12	2	3	..	5
xi. 29 ..	38	29	76.0	1,675	29	1
vii. 30 ..	35	8	23.0	610	8
viii. 30 ..	80	55	69.4	1,328	50	5	3
ix. 30 ..	108	80	74.1	1,240	65	15	3
x. 30 ..	53	43	81.1	3,219	39	4
viii. 31 ..	50	16	32.0	206	10	6	1
ix. 31 ..	27	16	59.3	1,036	8	8
x. 31 ..	25	20	80.0	3,692	18	2
xi. 31 ..	25	16	64.0	636	16
i. 32 ..	25	8	32.0	762	8
ii. 32 ..	25	9	36.0	471	9
iii. 32 ..	25	12	48.0	690	12
viii. 32 ..	75	13	17.3	198	5	8	1
ix. 32 ..	50	6	12.0	947	5	1	1
x. 32 ..	80	17	21.2	2,235	15	2	2
xi. 32 ..	50	18	36.0	1,884	17	1	6
xii. 32 ..	50	18	36.0	2,669	18	1
i. 33 ..	50	17	34.0	845	15	1	..	1	2
ii. 33 ..	50	12	24.0	300	12	1
iii. 33 ..	100	6	6.0	307	6	1
iv. 33 ..	80	4	5.0	175	3	1	1
v. 33 ..	86	9	10.4	224	7	2	1
vi. 33 ..	79	9	11.4	262	4	5	2
viii. 33 ..	85	5	5.9	140	3	2
ix. 33 ..	93	7	7.5	331	7	1
x. 33 ..	90	21	23.0	340	20	1	7
xi. 33 ..	85	20	23.5	605	20	2
xii. 33 ..	50	13	26.0	540	13	1

TABLE XI.

Results of blood examinations among the children of Dodai, 1927-1933.

Date.	Number examined.	Number found with parasites.	Parasite rate.	Average parasite count per c.mm. of blood.	NUMBERS FOUND WITH				
					M. T.	B. T.	Q.	Mixed B. T. and M. T.	Crescents.
x. 27 ..	33	4	12.1	3,630	3	1
vii. 28 ..	43	9	20.9	147	8	..	1
xi. 29 ..	50	37	74.0	2,019	34	3	1
vii. 30 ..	23	6	26.0	173	6
ix. 30 ..	30	14	47.0	1,070	14
ix. 31 ..	25	7	28.0	857	7	1
x. 31 ..	25	9	36.0	1,573	9	1
xi. 31 ..	25	9	36.0	2,642	9
i. 32 ..	25	13	52.0	538	13
iii. 32 ..	25	15	60.0	728	15
viii. 32 ..	49	7	14.3	114	5	2
ix. 32 ..	50	8	16.0	1,062	7	1
x. 32 ..	50	18	36.0	2,297	17	1	1
xi. 32 ..	50	26	52.0	1,441	26	3
xii. 32 ..	50	17	34.0	1,028	17
i. 33 ..	40	11	27.5	556	11	2
ii. 33 ..	50	8	16.0	337	8
iii. 33 ..	50	4	8.0	205	4	1
iv. 33 ..	50	7	14.0	240	6	1	1
v. 33 ..	56	8	14.3	542	5	2	..	1	..
ix. 33 ..	50	1	2.0	200	1
x. 33 ..	50	3	6.0	207	2	1	1
xi. 33 ..	50	8	16.0	312	8	4
xii. 33 ..	50	1	2.0	400	1

THE EFFECT OF PARIS GREEN DUSTING ON RICE CROPS

BY

LIEUT.-COLONEL G. COVELL, M.D., D.P.H., I.M.S.,
Late Assistant Director, Malaria Survey of India.

[21st March, 1935.]

PARIS GREEN has been in general use as an anopheline larvicide in almost every malarious country in the world for the last ten years. For a considerably longer period it has been applied as an insecticide on various crops, particularly in the United States of America.

From time to time complaints have been received from cultivators that their crops have been damaged by the application of paris green. Investigations have usually shown that these complaints have originated from one of the following reasons :—

(1) that the cultivators were genuinely under the impression that the application of paris green had resulted in a poorer crop.

(2) that there was a certain amount of ill-feeling against the local officials, with the result that any measure adopted by them was met by opposition.

(3) that there was a hope that compensation might be obtained from Government or local bodies as the result of the complaints.

Requests have been received on various occasions from workers in India who have met with opposition to paris green dusting for an authoritative statement as to the effect of this larvicide on crops, particularly on rice crops. In response to one of these requests, the writer sent out letters to various malariologists in India and in other countries, asking for their opinions on the subject. A search was also made for any references to the question in literature dealing with malaria control.

The results of these inquiries are given below, in the form of (A) extracts from the replies received from malaria workers, and (B) extracts from published articles.

A. EXTRACTS FROM LETTERS.

DR. ENGINEER, MEDICAL OFFICER, ANDHRA VALLEY POWER SUPPLY COMPANY, LTD., BHIVPURI:—

'Paris green 5 per cent in lime has been used on growing and fully grown rice for the past 3 years, and no complaints have been received from the owners.

The paddy as well as the grain were examined and found to be satisfactory. There have been no ill-effects under the following conditions :—

1. Paris green dusted on water inside the ricefields.
2. Paris green used on flowing water outside but going to the fields.
3. Paris green dusted on breeding places at the margins of ricefields causing clouds to go over the paddy by the breeze'.

RAI SAHIB J. D. BAILY, I.M.D., OFFICER-IN-CHARGE, SIND MALARIA INQUIRY:—

Has noted no ill-effects during work in Sind extending over several years. In one place the villagers stated that their rice crop was improved by the paris green dusting, owing to its preventing the appearance of insect pests.

DR. G. C. RAMSAY, PRINCIPAL, INDIA BRANCH, THE ROSS INSTITUTE:—

'As regards the question of the effect of paris green on rice, I personally have not seen nor heard of any ill-effects'.

DR. K. SATYANARAYANA, MEDICAL OFFICER, VIZAGAPATAM HARBOUR:—

'I tried to elicit this point from some ryots, some 2 years back, in the small areas in which I experimented with paris green. They did not complain of any deterioration of the paddy, or of bad effects from eating that rice.

I carefully watched the effect of paris greening one year ago in another small area, taken for experimental purposes. No bad effects are reported.

Five per cent paris green mixture was used, at the rate of 1 lb. of paris green to the acre'.

DISTRICT HEALTH ASSOCIATION, VIZAGAPATAM, RESOLUTION, NOV. 1933:—

'The Conference resolves that, so far as the knowledge of the members assembled here goes, oiled irrigation water or paris green has no evil effects on paddy or any other crops'.

DR. W. C. SWEET, CONSULTANT IN HEALTH, MYSORE STATE:—

'We have never had any complaints here about the effects of paris green on rice. The paddy fields must have received a considerable dose of the drug. The Director of Agriculture has never considered that this was harmful, and as the Farm is a paddy-breeding station, in which paddy is carefully weighed, analysed and graded, I am sure they would notice any possible effects.....I have never heard any reports from other countries in regard to any effect of paris green on rice.

One of the large landlords here objected strongly when we began the paris green work, on the grounds that it would injure his paddy. However, when we first stopped for the 6 months' interval, the same landlord came around and asked us to continue paris greening his paddy, even if we did nothing else, as the paris green acted as a control of parasites on the paddy'.

DR. L. C. COLEMAN, DIRECTOR OF AGRICULTURE, MYSORE STATE:—

'I have discussed the question of the effect of repeated doses of paris green on the paddy crops on our Nagenahalli Paddy-Breeding Station with Dr. V. K. Badami, Economic Botanist in administrative charge of the station.

Dr. Badami informs me that there has been no evidence whatever of any injurious effects from the paris green applications'.

CAPT. B. S. CHALAM, MALARIOLOGIST, E. B. RAILWAY:—

'Till about a month ago, I did not receive any complaints about the ill-effects of paris green dusted when the crop is in flower. Only recently there was a complaint from one of my control stations that the paddy turned brownish after one application of paris green, $2\frac{1}{2}$ per cent dilution in soft stone powder.

Apart from this I am not aware of any instances of the ill-effects of paris green on paddy'.

MALARIA MEDICAL OFFICER, SAVANTWADI STATE:—

'We have received no complaints about the bad effects of paris green on rice here. . . . On the contrary, the Agricultural Department advises to use this stuff, which is useful in destroying caterpillars, or such other pests, that destroy crops'.

R. A. SENIOR WHITE, ESQ., MALARIOLOGIST, B. N. RAILWAY:—

'The villagers put down every damage to paddy to paris greening, which I have in various places been told is the cause of damage which inspection clearly indicates to be really the result of fungoid diseases, of the plant bugs of the genus *Leptocoris* and Noctuid and Pyralid caterpillars, as well as attempts to grow paddy on quite unsuitable soil, of the results of shortage of water, and even of the burying of the crop by flood-carried silt'.

DR. H. F. CARTER, MEDICAL ENTOMOLOGIST, COLOMBO, CEYLON:—

'During our work with paris green we had only two complaints—one concerning paddy where the damage was subsequently shown to be due to the stem borer, and one regarding tobacco which was actually attacked by cut-worms'.

DR. L. L. WILLIAMS, IN CHARGE MALARIA INVESTIGATIONS, UNITED STATES PUBLIC HEALTH SERVICE:—

'We have never had any complaints of paris green or its alleged deleterious effect on any crop. . . . Paris green is very much used as an insecticide by our farmers'.

DR. R. K. COLLINS, ROCKEFELLER FOUNDATION, BULGARIA:—

'We have to treat a certain amount of rice each year, but have never had a complaint from any grower. The drug apparently does not affect the growth of the plant adversely'.

DR. E. W. WALCH, BATAVIA, DUTCH EAST INDIES:—

'I know only of one experiment during which a ricefield was treated with paris green during 35 days. When the experiment started the rice was 75 days old. No ill-effect on the crop was reported'.

Dr. J. W. SCHARFF, Malaya, Dr. I. J. KLIGLER, Palestine, Dr. L. W. HACKETT, Italy and Albania, and Dr. S. DE BUEN, Spain, replied that they had had no personal experience of dusting ricefields.

B. EXTRACTS FROM PUBLISHED ARTICLES.

BARBER, M. A., KOMP, W. H. W., AND HAYNE, T. B. (1926). Malaria in the Prairie Rice Regions of Louisiana and Arkansas: 'The quantity of paris green necessary to kill anopheline larvæ is not injurious to the rice plants'. *N.B.*—The authors mention that in one tract the rice was 30 inches tall, thick and blossoming. *U. S. A. Pub. Health Repts.*, **41**, 45, pp. 2527–2549.

CHALAM, B. S. (1930). Further Observations on Paris Green as an Anopheles Larvicide: 'The writer has had the opportunity of using paris green on a large scale for five years in Bombay and two years in Bengal. . . . In paddy fields there is no other preparation so far known to take the place of paris green. . . . The quantity of paris green used varied from $\frac{1}{4}$ to 2 lbs. per acre in the case of ricefields. . . . In the case of ricefields it may be stated in general as a result of these experiments that one pound of paris green per acre is a safe margin. . . . As regards anopheline control in paddy fields there seems to be no alternative to the use of paris green'. The author gives details of a series of experiments carried out in ricefields, and makes no mention of any ill-effects to the crop. *Rec. Mal. Surv. Ind.*, **1** 4, pp. 515–522.

KING, W. V., AND BRADLEY, G. H. (1926). Airplane Dusting in the Control of Malaria Mosquitoes: 'It was found from experiments that the leaves of the rice plants are very susceptible to burning when a large quantity of paris green is used, but the quantity necessary to kill anopheles larvæ did not prove destructive to the plants, and in a few small plot tests, where excessive quantities were applied, even severe burning of the leaves did not appear to kill the plants, or to prevent normal development of the grain'. *U. S. Dept. Agric. Circ. No.* 367.

NICHOLLS, L. (1927). The Use of Copper Aceto-arsenite (Paris Green) as an Anopheline Larvicide: 'Rice and other plants were grown in mud covered with a layer of water in the bottom of the tubs, and 2 grams of coir dust mixture containing 2 per cent of paris green (by weight) was placed on each square yard of water surface; and it had no deleterious effect on the plants'. The author conducted a series of experiments in ricefields, and makes no mention of any ill-effects to the crop. *Ceylon J. Sci.*, D. **2**, 1, pp. 21–30.

ROCKEFELLER FOUNDATION, ANNUAL REPORT FOR 1932, p. 69: 'Paris green. . . . has no ill-effects on rice or other crops'.

DISCUSSION.

As will be seen from a perusal of the above extracts, the evidence is overwhelming that, in the quantities in which it is used in antimalaria work, the application of paris green exerts no harmful effect on the rice crop. It is interesting to note that on several occasions owners of ricefields have actually stated that their crop was improved by the dusting, on the ground that it was inimical to certain insect pests.

An experiment to determine the effect of paris green dusting on rice crops was carried out by the Government of Bihar and Orissa in 1934, in the vicinity of Khurda Road Station, Puri District. In this area vigorous opposition to the application of the larvicide had been met with from the local cultivators, who alleged that their crops were being ruined by it.

Two similar plots were used in the experiment, one of which was dusted at regular intervals with paris green mixture, the other being left untreated as a control. The result of the experiment did not bear out the contention of the villagers that their crops were being ruined by paris green dusting; but the value of the experiment was largely vitiated by the fact that the amount of paris green applied was considerably less than one pound per acre, which is the amount usually applied in antilarval work.

The most important outcome of the experiment is contained in a note attached to the report by the Deputy Director of Agriculture, Orissa Range, Cuttack, who gave his opinion that during the flowering season paris green dusting is definitely harmful to the rice crop if done in the early morning when the flowers are open; whereas if it is done in the afternoon when the flowers are closed, no harm results.

SUMMARY.

(1) The general opinion amongst malaria workers who have had experience of paris green dusting of ricefields in various countries is that, in the quantities in which it is applied for antilarval purposes, this larvicide exerts no harmful effect on the rice crop.

(2) There is some evidence, however, that harm may result from dusting paris green over the open flowers of the rice plants. It is recommended that during the flowering season the application of paris green should be restricted to the afternoon, i.e., during the period when the flowers are closed.

OBSERVATIONS ON THE EMERGENCE OF ANOPHELINES.*

BY

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[8th March, 1935.]

INTRODUCTION.

WHILE working with the *Anopheles ludlowii* (*sundaicus*) enquiry of the Bengal Government, I have often been disappointed over the scanty yield from our elaborate campaigns to collect adults in certain places, although the number of larvæ found in these localities was plentiful. In such instances the rate of imaginal emergence is probably not in keeping with the larval rate. To investigate this point, a careful study of the phenomena associated with the actual emergence of the Anophelines was undertaken.

Workers in economic entomology realize that, with insects in general, successful emergence determines to a great extent the distribution of the species in any area. In the life of many insects, the act of emergence of the imagines is attended by obvious delicate implications, which are often taken advantage of by their natural parasites and enemies in attacking them. There may be other limiting factors as well, dependent on environmental subtleties which are imperfectly understood at present.

Further biological study of this nature is essential, in view of the growing demand for a better control of Anophelines. This entails a thorough understanding of all the phases in the life-history of the insect, however unimportant these may seem at first sight.

METHODS.

Larvæ in earthen dishes and glass jars were kept under constant observations, and, as soon as they pupated, were transferred to specimen tubes or larvæ bottles, the time of pupation being noted on the bottles or tubes. The times of emergence of the adults from these pupæ were then recorded along with the species and sex. Thus we were able to note the exact duration of the

* Read before the Indian Science Congress, 1934.

pupal stage and the time of emergence of the adults. Larvæ were reared in their natural breeding water as far as possible and fed on *Spirogyra*.

PROCESS OF EMERGENCE.

In the act of emergence, the pupal case bursts and a slit appears in the cephalo-thoracic region along the mid-dorsal line, through which the imaginal head first emerges. At this stage the head is bent downwards with the antennæ and mouth-parts lying close to the ventral body-wall. In its attempt to free itself from the pupal case, the imago makes violent jerking movements and gradually the imaginal appendages are liberated one after the other. While emergence is being accomplished the halteres vibrate rapidly. This movement of the halteres possibly helps the release of the imago by assisting the insect in its progress anteriorly out of the pupal case. The hind legs come out last of all.

The whole process described above usually takes about eight to ten minutes, though it is sometimes prolonged to fifteen minutes. The time taken depends probably on the amount of support afforded by the vegetation present in water. Where some supporting material is found, the imago gets a better foothold in pulling itself out of the pupal case. Absence of such support, as happens in the breeding jars, makes the act of emergence longer.

The imago immediately after emergence cannot fly. Its wings have first to dry and harden. Different mosquitoes may take different times in this respect. On the same date *A. annularis* took from one to two hours, while specimens of *A. barbirostris* on the other hand took eight minutes to half an hour (Table I).

TABLE I.

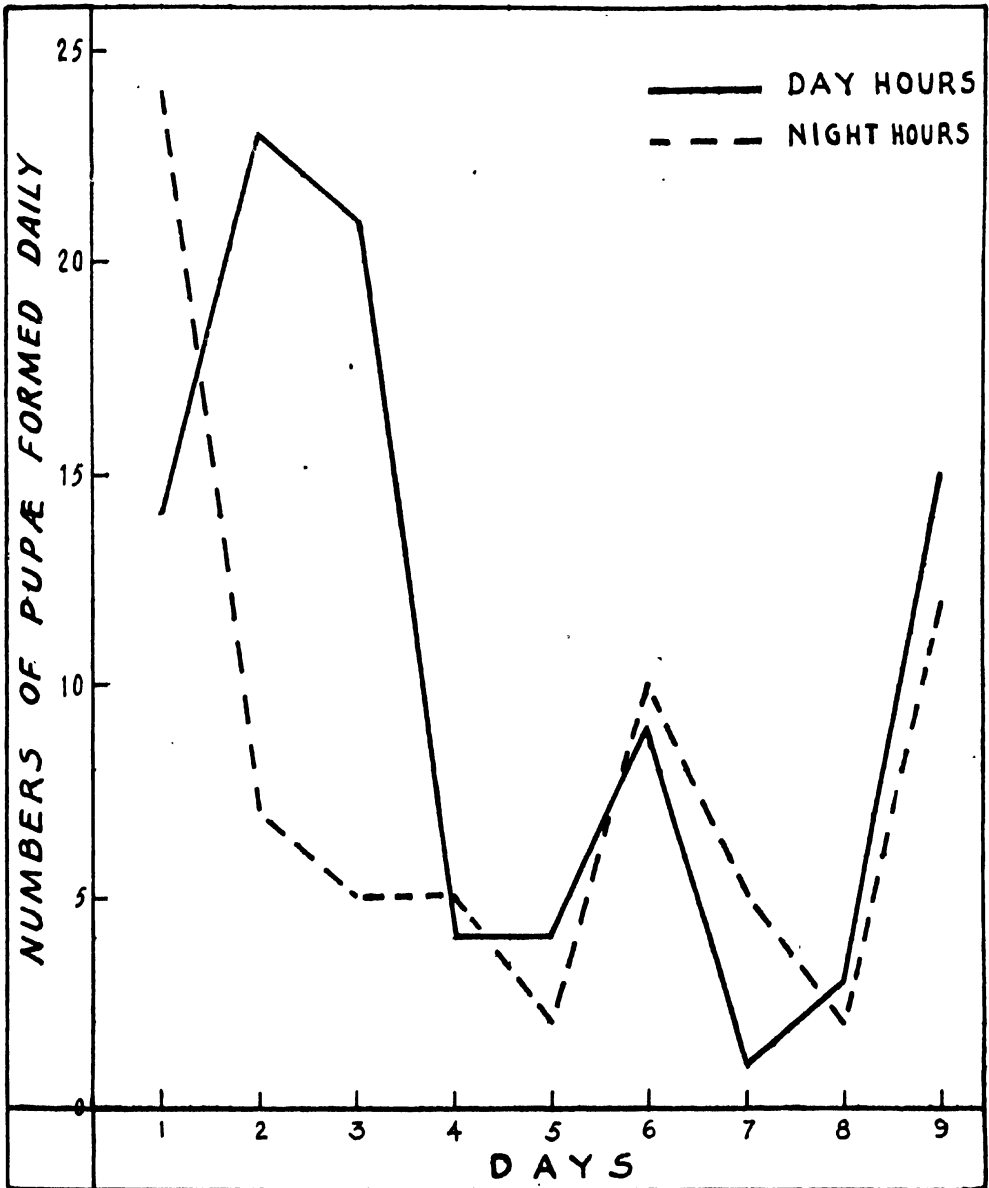
Date.	Species.	Time of emergence.	Time of actual flight.
11-10-33 ..	<i>annularis</i> .	3-12 p.m.	5-27 p.m.
" ..	<i>annularis</i> .	4-52 p.m.	5-35 p.m.
" ..	<i>barbirostris</i> .	5-10 p.m.	5-40 p.m.
" ..	<i>barbirostris</i> .	5-30 p.m.	5-38 p.m.
" ..	<i>barbirostris</i> .	5-32 p.m.	5-40 p.m.
16-10-33 ..	<i>hyrcanus</i> .	6-40 p.m.	6-55 p.m.

TIME OF PUPATION.

Pupation amongst the *Anophelines* occurred more often during the day than in the night, as commonly believed, and pupation in the morning is also not rare (Chart I). There is no regularity in the time of pupation in different species, nor is a particular species restricted to a particular time in the act of pupation. The time is variable both amongst the individuals of the same species and between the different species to some extent.

I have studied almost all the species that are common in deltaic Bengal, and have found that *A. varuna*, *A. annularis*, *A. hyrcanus*, *A. barbirostris*, *A. vagus*, *A. subpictus*, *A. stephensi* and *A. sundaicus* pupate during the course of the day. The majority of these day-pupating forms seem to prefer the late afternoon. Day pupation does not appear to be characteristic of any particular sex.

CHART I.



Time of pupation of anophelines.

DURATION OF THE PUPAL STAGE.

The duration of the pupal stage in the Anophelines differs in different species, and depends on the time of the year the mosquitoes are emerging,

the temperature factor being found to be of considerable importance in determining this. Thus, in summer *A. annularis* may occupy 26 hours only, *A. varuna*, *A. aconitus* and *A. philippinensis* about 27 hours, *A. vagus* and *A. subpictus* 27½ hours, *A. sundaicus* 28 hours and *A. hyrcanus* and *A. barbirostris* nearly 30 hours. In specifying the time I have given the minimum that might be occupied by the pupæ of the species concerned, and therefore is not absolutely constant to the species. The time occupied in the species being variable, quite often the duration of this stage in two or more species overlaps. In the cold weather, however, the pupal stage of all the species mentioned above usually takes a longer time, something like 46 to 48 hours or more. The duration in certain cases, especially in *annularis* and *hyrcanus*, has been noticed to be further prolonged when there was rain and consequently a further fall in the temperature in the winter season; *A. subpictus* and *A. vagus* may take 40 hours to complete their pupal stage as noticed in January 1934; and *A. culicifacies*, on which observations could only be made in December 1933, took for the pupal stage nearly 44 hours. The dependence of the duration of the pupal stage on the temperature and the season has been well brought out in the case of *A. sundaicus* (Table II). Similar tables could be drawn up for almost all the local species observed. Instances could often be found where certain individuals, even though of the same batch, metamorphose to the imaginal stage sooner or later than their fellows. I have also noticed that, while extraneous disturbance may, under exceptional circumstances, hasten the pupation of the preparing larvæ, the pupæ are not usually affected by any change or disturbance in the habitat.

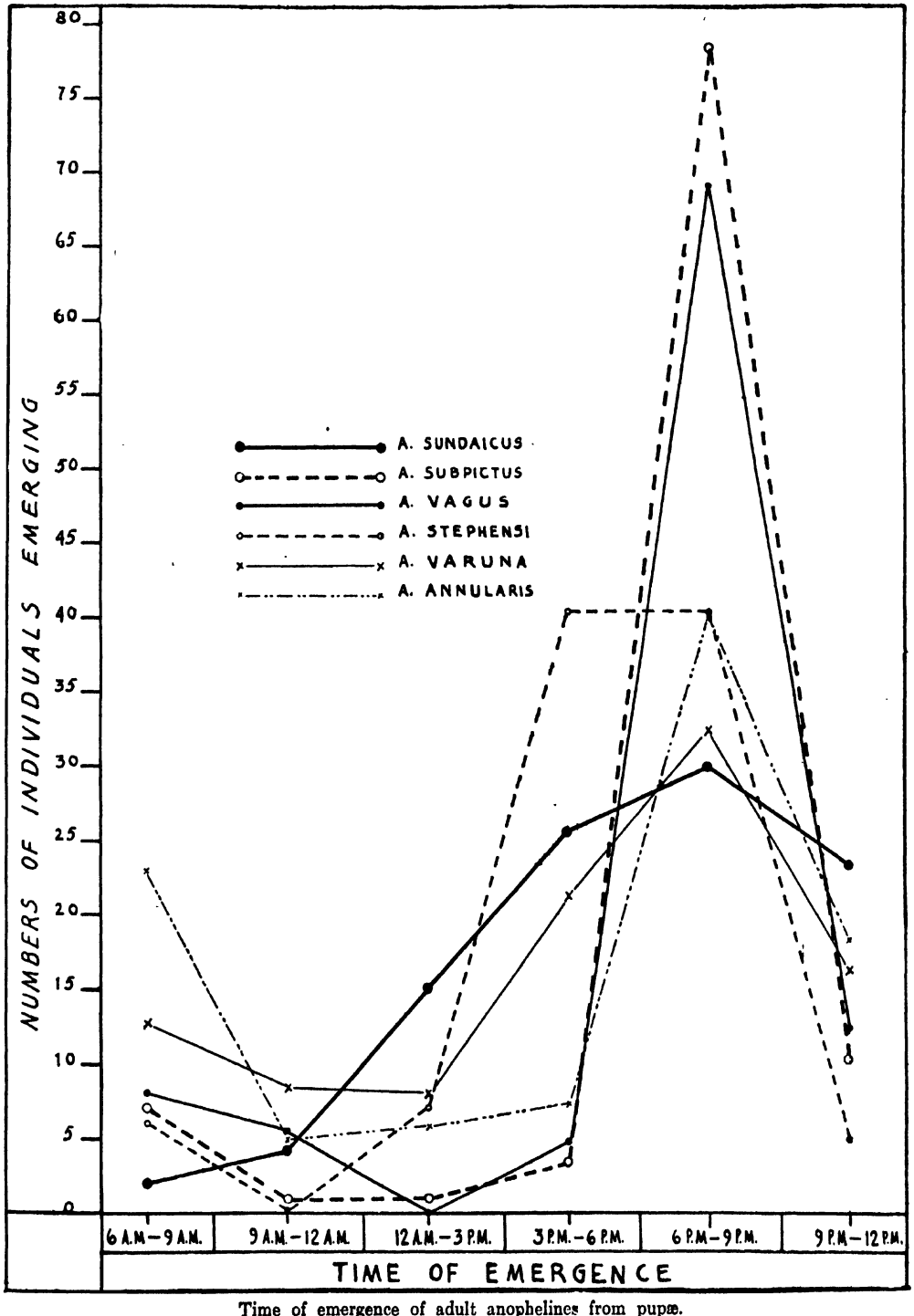
TABLE II.
Duration of the pupal stage of A. sundaicus.

TIME OF PUPATION.		Sex.	Number.	TIME OF ADULT EMERGENCE.		Temperature, °F. Max-Min.	Duration of pupal stage.
Date.	Hour.			Date.	Hour.		
8-11-33 ..	1 p.m.	♀	2	9-11-33	10-30 p.m.	83-81	33½ hours.
12-11-33 ..	11-45 a.m.	♀	1	13-11-33	11-45 p.m.	82-80	36 "
14-12-33 ..	3 p.m.	♀	1	16-12-33	12-30 p.m.	75-73	45½ "
18-12-33 ..	12-15 p.m.	♀	1	20-12-33	10 a.m.	75-71	46 "
16-1-34 ..	night	♀	1	19-1-34	6-30 a.m.	72-50	above 48 "
30-1-34 ..	3 p.m.	♂	3	1-2-34	2-15 p.m.	76-68	48 " approx.
16-6-34 ..	3-4 p.m.	♂	2	17-6-34	6-10 to 8 p.m.	93-87	27-28 " approx.
19-6-34 ..	11 a.m.	♂	1	20-6-34	7-10 p.m.	89-86	32 "
21-6-34 ..	2 p.m.	♀	1	22-6-34	6-40 p.m.	91-87	29 " approx.

TIME OF EMERGENCE OF IMAGINES.

In our experiments the *Anophelines* usually emerged towards the dusk. Emergence may however take place at any part of the day, at least under laboratory conditions at the ordinary room temperature. I have observed from a study of some thousands pupæ that the emergence is largest between the hours 6 and 9 in the evening, representing nearly 46 per cent of the complete experiments (Chart II). The rate of emergence is also high during the hours 9 p.m. and midnight (12 p.m.), and towards the morning.

CHART II.



There is no regularity in the time of emergence with the different species of Anophelines. In the same day a certain proportion of a particular species has been found to emerge in the day time and the rest during the night, just as it does not follow that the larvæ of apparently same age should always pupate together.

The emergence curve varies in the different species, and may depend on the season. It may also vary from year to year. Thus in June the rate of emergence between the hours 6 p.m. and 9 p.m. reached nearly 89 per cent out of a total emergence of 289. In the months of September to December, the emergence rate may attain 40 to 50 per cent at these hours. The species *A. sundanicus*, *A. subpictus*, *A. vagus*, *A. barbirostris*, *A. ramsayi*, *A. annularis*, *A. philippinensis*, *A. varuna* and *A. aconitus* were found mostly to emerge between the hours 6 p.m. and 9 p.m. The peak of the emergence of *A. hyrcanus* occurs during the next three hours, between the hours 9 p.m. and midnight. The specimens of *A. culicifacies* that I was able to observe emerged during the late night hours; the species is a rarity in the area as noted by Senior White (1934). With *A. stephensi* the intensity of emergence reached its maximum long before dusk, but the high level was maintained up to 9 p.m.

Incidentally it was noted that, amongst *Culex fatigans*, the majority emerge during the hours 6 and 9 in the evening, although stray emergence has been known to occur during any hours of the day, and a certain percentage may also emerge towards the morning hours. Adults of *Aedes aegypti* on the other hand mostly emerge towards the late afternoon, next suitable time for the emergence being the morning hours (Chart III).

CASUALTIES IN IMAGINES DURING EMERGENCE.

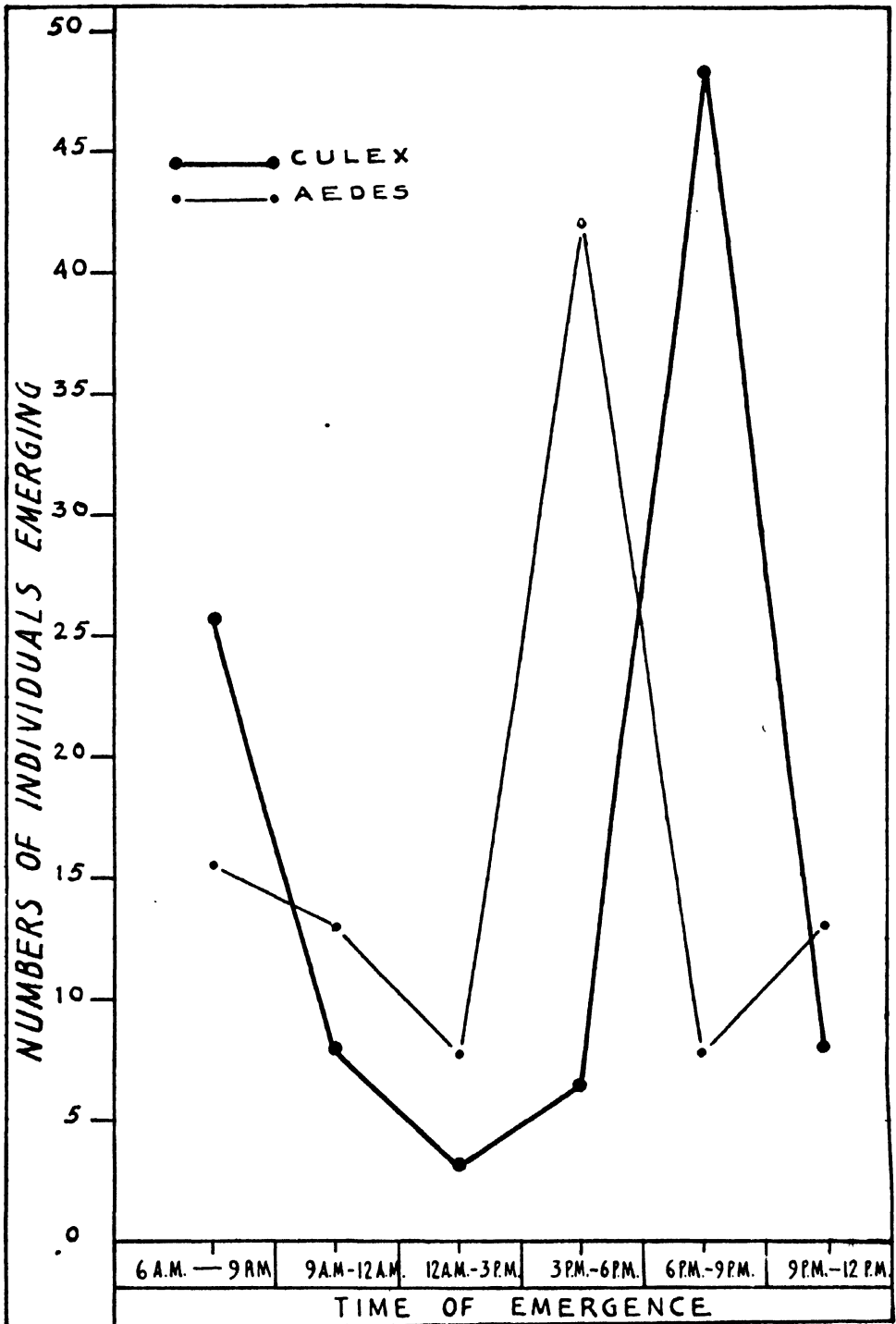
Emergence like pupation is usually attended with a considerable mortality, at least under laboratory conditions. Half-emerged adults are often found drowned in the breeding jars. In some there are certain malformations either in the wings or legs. The adults under such circumstances are unable to free themselves from the pupal case and meet a watery grave. Such mortality in batches of pupæ sometimes reached as high as 40 to 50 per cent. The pupæ of *A. vagus*, *A. varuna*, *A. annularis* and *A. hyrcanus* were more often involved in this mortality. *A. subpictus* pupæ were more tolerant in this respect, the mortality rate in this species being less than 10 per cent. The total mortality of all the species comes to about 22 per cent.

The temperature of the water may affect the mortality. Thus on several occasions I have seen that a temperature of 36°C. to 38°C. inside an incubator has proved fatal to the pupæ. Once only at this temperature did the adults actually emerge in less than 24 hours, but they ultimately were killed by the heat of the incubator. The sexes were not differently affected, as in the last instance males and females were equally represented.

Excess of cold also is likely to bring about death to the pupæ. If the temperature of the water containing the pupæ be lowered to below 10°C. by placing the jars in an ice chamber, the activities of the pupæ are stopped and they ultimately die within a day.

Ants have often been found to prey on the pupæ.

CHART III.



Time of emergence of adult *Culex* and *Aedes* from pupæ.

SEX RATIO.

The present study also throws some light on the sex ratio of the mosquitoes emerging under laboratory conditions. The proportion of sexes as recorded for the different species is shown in a tabular form and expressed as percentages (Table III).

The ratio has been worked out from the sum total of the emergences, which varied considerably in the different species as observed at different seasons of the years 1933 and 1934. In the experiments water from natural breeding places was utilized as far as possible and the larvæ were supplied with a plentiful supply of *Spirogyra*.

Table III shows that in most of the species females are in excess of males, forming nearly 60 per cent of the total emergence, in other words the sexes are generally represented in the proportion of two males to every three females. In this respect different batches behave differently. When the broods studied under each species are considered separately, it is not unusual to find instances where the sexes appear in equal proportion, or in some batches the males predominate, although the great majority of batches show excess of females (Charts IV, V and VI).

The fluctuations in the sex ratio, at least in some species, possibly depend on the weather conditions. There is some evidence that in dry weather the males predominate; this was especially noticeable in *A. sundanicus* during the months February to June 1934. But when the rains set in and, in cool weather, the proportion becomes reversed, the female emergence then being much in excess.

DISCUSSION.

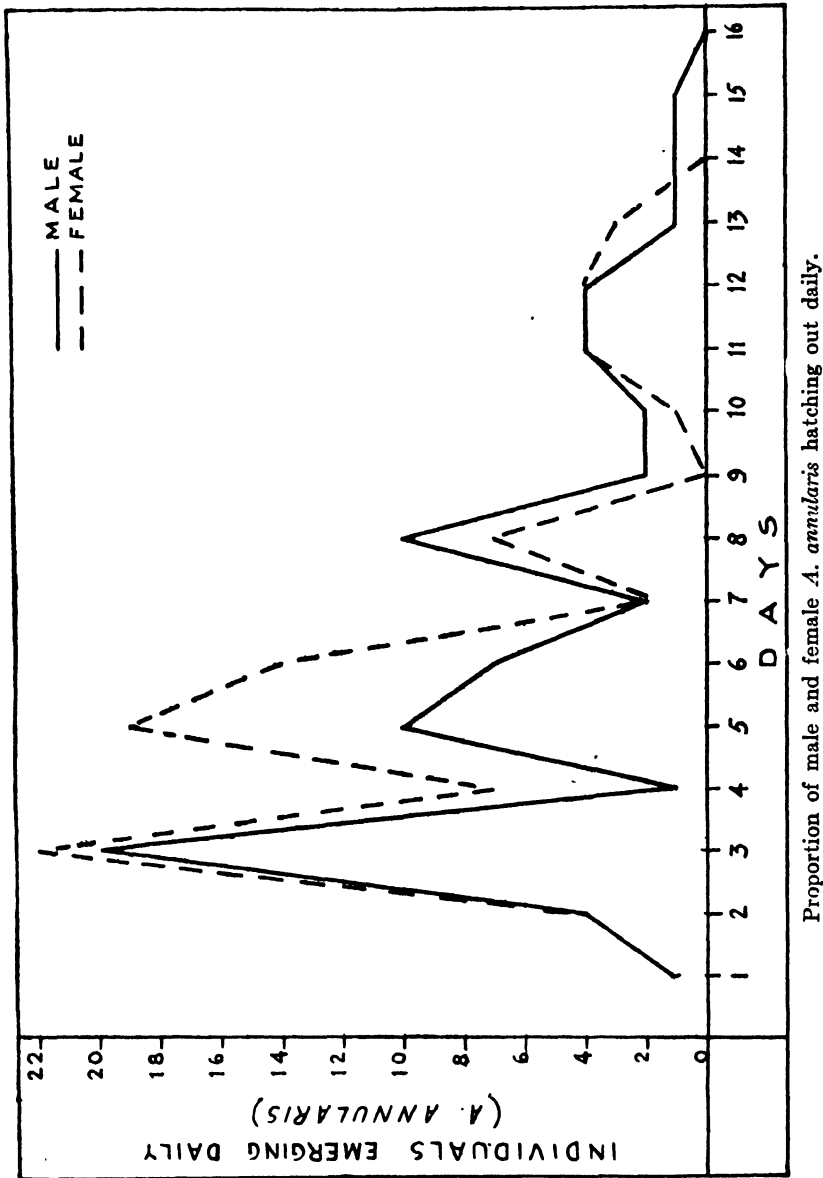
Lamborn (1922) has dealt with the phenomenon of emergence in *Anophelines* in brief. But his observations somewhat differ from mine. Lamborn states that 'in the great majority of instances pupation took place during the night', day pupations amongst *Anophelines* being only sparsely met with by him. He records a few stray instances of day pupation in the species *vagus*, *subpictus*, *ludlowii*, *karwari*, *maculatus*, *hyrcanus*, *barbirostris*, *fuliginosus*, *kochi* and *tessellatus*. While pupation during the night is not so infrequent, I have found that pupation amongst the *Culicidæ*, inclusive of some of the species mentioned by him, occurred more often during the course of the day. The reason of the anomaly is not obvious unless it is due to the climatic variations of Malaya where Lamborn worked. The duration of the pupal stage differed in the different batches, as well as within the various species to a certain extent. The length of the pupal phase appears to depend considerably on the weather conditions. While in summer this stage may occupy only 26 hours (or even less) in the cold weather the duration may be extended to 48 hours. This dependence of the *Anopheline* pupæ on weather conditions probably has given rise to the apparently diverse findings amongst authors. Lamborn (1922), for instance, states that this stage lasts from 36 to 48 hours, no prolongation of this time being ever noticed by him. Macfie (1915) working with *Stegomyia* found that the pupal stage would often last three days, and again Young (1922) suggests that it may take even five days. Howard, Dyar and Knab (1912), and more recently Christophers (1933), also have suggested that at low temperature conditions the period may be

TABLE III.

Species.	Months covered in the observations.	Actual numbers of emergences recorded.	SEX RATIO.	
			Male (per cent).	Female (per cent).
<i>A. eundatus</i> Roden.	October 1933 to February 1934; April 1934 to June 1934; August and September 1934	303	43	57
<i>A. stephens</i> Liston	November 1933; October 1934	92	35	65
<i>A. subpictus</i> Grassi	September 1933 to February 1934; April to June 1934	337	40	60
<i>A. vagus</i> Donitz	October 1933 to March 1934; June and September 1934	151	36	64
<i>A. barbirostris</i> Van der Wulp	September 1933 to March 1934	88	38	62
<i>A. hyrcanus</i> var. <i>nigerrimus</i> Giles	October 1933 to March 1934; June and September 1934	205	45	55
<i>A. ramsayi</i> Covell	October and November 1933; February 1934	46	33	67
<i>A. annularis</i> Van der Wulp	October 1933 to March 1934; June, July and September 1934	523	41	59
<i>A. philippinensis</i> Ludlow	June 1934	14	50	50
<i>A. varuna</i> Iyengar	October 1933 to February 1934; September 1934	119	40	60
<i>A. aconitus</i> Donitz	November and December 1933; February 1934	26	25	75
<i>A. culicifacies</i> Giles	December 1933	12	50	50
<i>Culex fatigans</i> Wied.	October 1933 to March 1934	166	53	47
<i>Aedes aegypti</i> Linn	March, September and October 1934	114	42	58

prolonged to three days; the latter author cites *A. gigas* a hill species taking eight days in cold weather.*

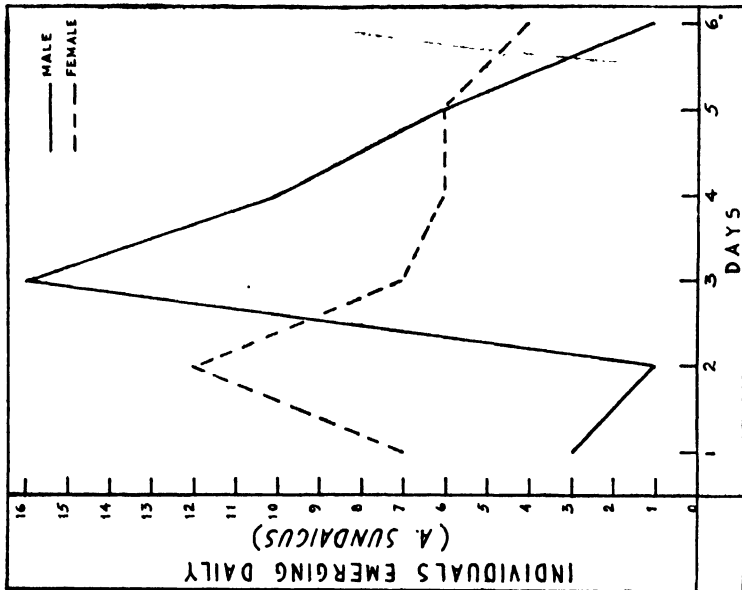
CHART IV.



*I have in my records a single observation on *A. insulaeflorum* from the submontane region of the Himalayas taking three days to complete its pupal stage, when brought to my laboratory in the month of February.

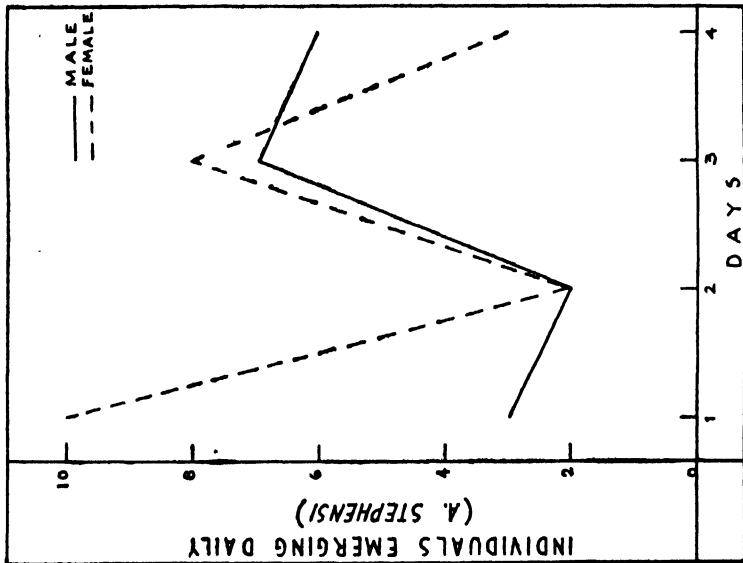
I have observed several hundreds of actual emergences and have found that the eclosion may take place during any hour of the day, but is generally most brisk towards the dusk. Grouping the emergences in periods of three

CHART VI.



Proportion of male and female *A. sundacicus* hatching out daily.

CHART V.



Proportion of male and female *A. stephensi* hatching out daily.

hours the imagines seem to prefer the hours six to nine in the evening for this act. Lamborn (1922) states that the emergence usually takes place before

11 p.m., an observation not very different from mine. The twilight activity of the Anophelines as observed by Howard, Dyar and Knab (1912) and Ludlow (1914) is therefore quite conceivable, since the maximum emergence takes place during this time of the day. Howard *et al.* (1912) doubt the truth of the belief in the nocturnal habits of most Anophelines. These authors hold that the activities of Anophelines are confined to the twilight of evening and early morning. That the Anophelines can emerge in the morning hours has also been shown by me. *Aedes aegypti* and the allied species on the other hand are normally diurnal in habits, and the maximum emergence of these mosquitoes has been noted to occur about three o'clock in the afternoon, which happens to be the period of its greatest activity as suggested by Theobald (1901).

Mehta (1934), in discussing the sex ratio of the Anophelines, suggests that the sexes appear in equal proportion or the females slightly predominate, a view essentially agreeing with that of Christophers (1933), and that the ratio does not depend on the food supply. I have found that the females are largely the predominating sex, forming nearly 60 per cent of the total emergence when a large number of observations are considered. When we examine observations of Mehta (1934), and take a complete account of all the emergences in his various experiments in the laboratory with *A. subpictus* and *A. annularis*, it becomes evident that the sexes, males to females, are represented in the proportion of 46 to 54 for *subpictus* and 44 to 56 for *annularis*. Although I have not gone into the subject of the effect of food supply in the determination of sexes in the Culicidae, it strikes me from Mehta's observations that the food supply may have some influence in determining the ratio. Mehta's four experiments on *subpictus* (producing 72 males and 85 females) with *Spirogyra* as food for the larvæ, when considered *in toto*, give the ratio of males to females as 46 to 54. The same species in his other two experiments, when given a change of diet in the form of macerated house flies, produced 99 males and 107 females, the sex ratio in the case being distinctly raised to 49 to 51. Again in his experiments with *A. annularis*, when the larvæ were reared in distilled water with yeast as food, the sex ratio was found to be 38 to 62, but when the water from a natural breeding pool (undoubtedly containing some microscopic organisms in which larvæ feed in nature) was substituted for distilled water the ratio went up to 46 to 54. May it not be that a protein diet, as supplied through the fly-food and the plankton fauna present in the water of a natural breeding pool, accounts for the increase in the proportion of males?

I have observed, especially in the case of *A. sundaicus*, that the domination of one sex over the other may depend on the seasonal conditions. In the dry weather for instance, it is usual to find a great increase in the male emergence, as has also been found true for *A. annularis* and *A. culicifacies* (emerging during the period March to June) studied by Mehta (1934). I am able to confirm his findings that, in the order of hatching, either sex may appear first or the sexes may appear simultaneously.

SUMMARY.

The actual process of emergence of the imagines from the pupal stage has been described. The whole process takes about eight to ten minutes. Pupation in the Anophelines more often occurs in the day than in the night. Actual observations were made on the species *A. varuna*, *A. annularis*,

A. hyrcanus var. *nigerrimus*, *A. barbirostris*, *A. vagus*, *A. subpictus*, *A. stephensi*, *A. sundaicus*, *A. philippinensis* and *A. culicifacies*.

The duration of the pupal stage in the different species is somewhat variable. Time taken varies from 26 hours in summer to 48 hours in winter, and is found to depend on the seasonal conditions and temperature factor.

The imagines emerge mostly between the hours 6 and 9 in the evening. Emergence may however take place in the day-light and towards the early morning.

The mortality rate on an average in the emerging adults was found to be 22 per cent in the laboratory. Pupæ succumb to extremes of temperature and a considerable number are preyed upon by the natural enemies like ants, etc. Malformations in the locomotory organs of the emerging adults also bring about death to some.

The sexes are represented in the proportion of 2 males to 3 females in a whole year's emergence. Batches behave differently in this respect, and the climate also plays a rôle in the regulation of the proportion. Dry weather impairs the emergence of the females.

In conclusion I wish to acknowledge my indebtedness to Lieut.-Col. A. D. Stewart, C.I.E., I.M.S., for his readiness to discuss matters with me. To Dr. R. B. Khambata, Director of Public Health, Bengal, and Dr. S. N. Sur, I am grateful for the facilities they have afforded me. Lastly I have to thank my successive Laboratory Assistants for their readiness in recording actual emergences whenever called upon to do so, even during night hours at considerable sacrifice to their personal comfort.

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QUANTITATIVE CHANGES IN THE PROTEINS OF THE BLOOD SERA OF MONKEYS INFECTED WITH MALARIAL PLASMODIA.

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I. INTRODUCTION.

A QUANTITATIVE study of the proteins of the blood serum or plasma in various diseases has supplied information of much scientific interest. It has been recorded by Epstein (1912, 1913),* by Linder, Lundsgaard and van Slyke (1924),* and by Lloyd and Paul (1929)* that in normal persons the ratio of globulin to albumin in the blood serum varies within narrow limits. In many pathological conditions, however, this ratio may show a wide range of variation.

In nephrosis and nephritis, the plasma proteins have been estimated by a number of workers, and the appearance of oedema in these and other pathological conditions has been reported to be connected closely with a decrease in the total proteins and in the albumin fraction (Moschcowitz, 1933). It has been found by some observers that oedema generally occurs when the percentage of total proteins falls below 5.5 and that of albumin below 2.5 (*vide* Peters and van Slyke, 1932).

The variations in serum proteins have been studied in a large number of different diseases. Among protozoal infections, several workers have directed their attention to kala-azar. It has been found by Brahmachari (1917), by Wu (1922), by Ray (1924, 1927), by Lloyd and Paul (1928), and by Lloyd, Napier and Paul (1929) that, when a kala-azar infection is of several months' duration, there is a marked increase in the ratio of globulin to albumin in the blood serum. Lloyd and Paul (1928) record that in untreated cases of this disease there is a fall in the albumin percentage with a rise in the globulin, mainly due to euglobulin, and in the globulin/albumin ratio, which may exceed unity.

Lloyd, Napier and Paul (1929) studied especially the effects of treatment upon the blood proteins in this disease, and consider that the efficacy of various antimonial preparations can be gauged by the typical serological changes which occur during the progress towards cure. These changes were mainly a rapid rise in albumin and a fall in globulin, especially in euglobulin, with a resultant return of the globulin/albumin ratio towards normal.

II. PREVIOUS WORK ON THE SERUM PROTEINS OF THE BLOOD IN MALARIA.

Various workers have investigated the effects of malarial infections upon the blood proteins of human patients. Much of this work has been connected with the question of the rate of sedimentation of the red blood cells in relation to the fibrinogen of the plasma. Several persons have, however, studied the serum proteins alone, and, as will be seen, there is a considerable amount of agreement between the findings recorded.

Petersen (1926) reports that in malarial cases there was a diminution of the blood proteins during the rigor and the rise of the fever, and, on the contrary, an increase as soon as the fever was established and when it fell to normal.

Radošević and Ristić (1926) studied the proteins of the blood *plasma*, with special reference to their relationship to the rate of sedimentation of the red blood cells. They state that, at the height of each paroxysm, there was an increase in fibrinogen in the plasma with an absolute decrease of albumin, but, during the apyrexial period, the change was reversed. The administration of quinine or neosalvarsan to such malarial patients

* Epstein and Linder *et al.* used the micro-Kjeldahl method, while Lloyd *et al.* relied upon the refractometric one in the estimation of the protein fractions.

produced a rapid return to normal conditions, corresponding sufficiently to the disappearance of the parasites.

Weichmann and Horster (1926) investigated the protein content of the serum in cases of inoculation malaria. They report that, during the incubation period of the infection, the protein content fell at first and then rose. With the appearance of fever, the proteins again fell, but this fall preceded the appearance of pyrexia. During the incubation period, the serum globulin was found to be in excess, the serum albumin fell and, with these changes, the globulin/albumin ratio rose. The serum albumin generally fell with the onset of pyrexia, and did not increase immediately upon the disappearance of fever. With the occurrence of convalescence, these proteins tended to return towards normality.

Lloyd, Napier and Paul (1929) report that the characteristic protein graph in malaria exhibits a low total protein, the albumin being greatly reduced and the globulin slightly. Under the influence of quinine treatment, both the latter curves were found to rise rapidly to normal.

Lloyd and Paul (1929) studied the serum protein changes in the malarial attacks of 8 patients with histories of very chronic infections. In the bloods of four of these, *P. falciparum* was found at the time of the primary examination, and in the others *P. vivax*. These patients were studied over long periods, during and after the termination of short courses of quinine treatment. In six of them single protein estimations were made immediately before treatment commenced. The following characters were shown by the serum before treatment :—

(i) a low protein content; (ii) this fall in proteins was almost entirely due to a very marked reduction in the albumin fraction; (iii) some reduction in the total globulin figure was also seen; this fall was always much less than the fall in albumin, and in some cases was very slight or absent; (iv) the euglobulin fraction was always raised, usually being about double the normal figure; (v) because of the euglobulin rise, the pseudoglobulin was correspondingly reduced where the total globulin was unaltered, and still further reduced if the total globulin was subnormal; (vi) because of the marked fall in albumin, the globulin/albumin ratio was raised considerably from normal (0.66), usually to slightly below, but in no case exceeding unity; and (vii) in one case of malignant tertian malaria, which relapsed during the period of post-therapeutic observation, the albumin curve showed a drop and that of globulin a slight rise during this attack.

These workers concluded that the fall in albumin, which was found to last beyond the fall of the fever, was not a function of the actual temperature, nor was the fall in total proteins, which resulted from this marked decrease in albumin. It did not matter whether the blood was taken at the height of the rigor, or in the interval between paroxysms, the albumin was always very low in their cases, and the euglobulin about twice the normal amount. The extent of the albumin reduction appeared to have some connection with the intensity of the infection.

As a result of their observations upon the effects of quinine treatment, they report that :—

(i) the administration of this drug was followed by a very rapid return of the total serum proteins to normal (there was an interval, however, between the defervescence of fever and the return to normality); (ii) the steepness of the rise in the albumin curve was particularly striking; the rise was at first rapid, but later became slower as the protein fraction approached normality; (iii) quinine treatment appeared to have little effect upon the globulin value in the majority of cases: 'only in those cases where it is depressed initially does the globulin value rise under the influence of quinine' (the graphs, however, show a slight rise in most cases); and (iv) the raised globulin/albumin ratio falls with treatment.

Ogden (1930) states that in untreated dementia paralytica there is an apparent increase of serum protein with a corresponding globulin increase. Following upon malarial therapy of such cases, there was no discernible increase in serum protein, but the albumin/globulin balance was disturbed with an apparent increase of globulin.

Dumolard *et al.* (1930) estimated the proteins in the sera of 10 natives of Algeria, suffering from chronic malaria. The patients had splenic enlargements and marked anæmia. These workers report a constant, and often very marked, diminution in the total proteins of the sera of such cases, affecting both the albumin and the globulin. As a rule the fall in the albumin was much more marked than in the globulin, and as a result the normal globulin/albumin ratio was reversed.

Bordes and Nguyen-Van-Lieng (1931) studied the same problem in 21 cases of chronic malaria among Annamites. Of these patients, 1 was infected with *P. vivax*, 19 with *P. falciparum* and in 1 the species of parasite was doubtful. These observers report the following changes :—(i) the total proteins of the serum were generally very slightly below the normal average, and, except in one case (*P. falciparum*), the differences were slight; (ii) the percentage of albumin was usually distinctly diminished and that of globulin increased; and (iii) this resulted in a notable lowering of the albumin/globulin ratio, which was in 14 cases below the lowest normal ratio observed. They were unable to confirm the enormous fall in total proteins reported by Dumolard *et al.* (1930).

A case of malaria, aged 30 years, with an enlarged and painful spleen, unaffected by intramuscular injections of quinine, was studied by Benhamou (1932). This patient was splenectomised and had a relapse due to *P. vivax* about 3 weeks after the operation. A blood examination made 8 days before the splenectomy showed an albumin/globulin ratio of 1·0. An estimation of the serum proteins made 2 months later revealed a fall in the total proteins from 70 gm. to 54 gm. per 100 c.c. serum, a fall in albumin from 35 gm. to 18 gm., while the globulin showed practically no change (35 gm. and 36 gm.). The result was that the albumin/globulin ratio then was about 0·5.

Tareev and Gontaewa (1933) examined 35 cases of acute attacks of malaria during either fresh infections or relapses of natural infections, and also 8 cases during the course of chronic ones. They found that the albumin/globulin ratio was lowered in the majority of cases, and that this fall was proportionate to the severity of the attack. During the apyrexial period after an attack, this ratio tended to rise again towards the normal value. Treatment appeared to assist this return to normality.

Fifteen cases of inoculation malaria were also studied. In these patients, Tareev and Gontaewa found that the percentage of albumin in the serum fell markedly, while the globulin was lowered but slightly, if at all. They also found the albumin/globulin ratio was lowered in most cases of malaria, and in inoculation malaria they considered that it fell progressively with the number of rigors experienced by the patient. With the termination of the fever the ratio returns to normal. (The protocols of the original paper suggest that in natural infections the globulin/albumin ratio rose with an acute attack and fell after it was over. The longer the interval between attacks, the greater seemed the tendency to return to normal. Treatment appeared to help this return towards normality. Chronic latent infections, examined some months after the last attack, showed that the globulin/albumin ratio had returned to about normal.)

James (1933), in speaking of inoculation malaria in the treatment of mental cases, says that an attack of malaria causes marked reduction in the total proteins. This reduction is chiefly due to a fall in albumin, but there is also some reduction in the amount of total globulin and pseudoglobulin, but the euglobulin is always raised.

The researches into human malarial infections reported above indicate that malarial attacks cause a distinct fall in the total proteins of the blood serum, which fall is most marked in the albumin fraction. There seems, however, to be some difference of opinion regarding the effects produced upon the globulin fraction, but there appears little doubt that there is a marked rise in the globulin/albumin ratio. The evidence also suggests that, when the attack has passed over, there is a marked tendency for the proteins to return gradually to their normal values. In chronic infections, in the absence of acute attacks, while the total proteins and the albumin fraction may be decreased and the globulin/albumin ratio raised, these changes are less marked than in acute infections.

Some of the observations suggest that the development of such protein changes may be proportionate to the intensity of the infection at the time the blood is examined. It appears probable, also, that the development of these protein changes may be prevented to some extent, and the rapidity of their return to normality may be markedly accelerated, by anti-malarial treatment.

A number of these reports have been based upon investigations made on the blood sera of patients receiving malarial therapy. In this connection,

it must be noted that Ogden (1930) points out that in untreated cases of dementia paralytica, there is already a disturbance of the blood proteins—an apparent increase of serum protein with a corresponding globulin increase. Under these conditions of disturbed protein balance, experiments carried out on such patients cannot be accepted as conclusive proof of the sequence of events which may occur in a malarial infection in a previously healthy normal individual.

Again it will be noticed that a very large number of the reports have been based upon single examinations of the bloods of untreated patients, and, when multiple examinations have been made, these have been mainly during or after treatment. Such isolated observations of untreated cases, many of whose previous histories were vague, are sometimes difficult to fit into their proper places in the natural progress of the course of the disease. A more continuous series of observations, uninfluenced by treatment or intercurrent disease, appeared necessary to obtain a true picture of the sequence of events.

A series of human patients fulfilling these ideal conditions would be almost impossible to obtain. We have therefore conducted our researches on monkeys infected with simian malaria. These were all healthy animals before inoculation, their malarial histories from the date of primary infection were known intimately, and treatment could be withheld when necessary. It, therefore, appeared to us that much more reliable information could be obtained under these conditions.

III. EXPERIMENTAL METHODS.

The common brown monkey of northern India, *Silenus rhesus*, has been used in all our experiments. These animals were in most cases young specimens infected by blood inoculation with different species of *Plasmodium* originally derived from the Malayan monkey, *S. irus* (*vide* Sinton and Mulligan, 1933; Sinton, 1934). Infections with the following three species of *Plasmodium* were studied.

(i) *P. knowlesi* Sinton and Mulligan, 1932, a parasite showing a quotidian periodicity;

(ii) *P. cynomolgi* Mayer, 1907, a parasite with a tertian periodicity; and

(iii) *P. inui* Halb. and Prow., 1907, a parasite with a quartan periodicity.

Blood was collected into sterile tubes from a prominent vein in the leg of the experimental animal. On the next morning the serum was separated from the clot, and centrifuged if it still showed any turbidity. The clear supernatant fluid was used for the estimations.

The serum proteins were estimated by the micro-refractometric method of Robertson (1915), using an Abbé-Zeiss refractometer. That protein fraction of the blood serum, which was precipitated by half saturation with ammonium sulphate, was termed 'globulin'.

As some workers have expressed doubt as to the reliability of this refractometric method, we have checked our results by the micro-method of Kjeldahl in several instances. As would appear from the following data, the results obtained agreed fairly closely in the two methods:—(i) Monkey No. 64. Total protein by Kjeldahl's method—6.9 per cent, by Robertson's method—6.85 per cent. (ii) Monkey No. 142. Total protein by Kjeldahl's method—7.4 per cent, by Robertson's method—7.5 per cent. (iii) Monkey No. 145.

Total proteins by Kjeldahl's method—6·5 per cent, by Robertson's method—6·4 per cent.

The question as to whether the serum globulin is a mixture of the so-called euglobulin and pseudoglobulin fractions, or is a single substance, has not yet been settled satisfactorily. Fractional precipitation by ammonium sulphate, or by sodium sulphate, yields samples of globulin which differ from each other in their solubilities. Sørensen (1925) and Adair (1926) have also found a somewhat higher osmotic pressure for pseudoglobulin than for euglobulin. Using the ultra-centrifugal method, Svedberg *et al.* (1928, 1930) have determined the molecular weight of globulin at its iso-electric point, so as to eliminate the effect of the Donnan-membrane potential, and have found that it consists of only one kind of molecules. It has, therefore, been suggested that the so-called pseudoglobulin and euglobulin are artificial products, formed during the fractional precipitation with electrolytes. In view of the doubtful existence of two distinct kinds of globulins, only the total globulin has been estimated in our experiments.

A series of estimations of the proteins of the blood serum have been made in monkeys at various stages of their malarial infection. Some of these were studied during the primary acute attack, others during relapses, either of this attack or during the course of prolonged chronic infections.

IV. RESULTS OF THE EXPERIMENTS.

(1) NORMAL MONKEYS.

We have been unable to find any records of investigations into the amounts of the different proteins present in the sera of normal monkeys. It was, therefore, necessary as a preliminary step in our work, to obtain some idea of the variations which occurred in healthy animals, for comparison with those found in infected ones. The total proteins, the albumin, and the globulin were estimated in the sera of eight normal monkeys.

The results of this work are shown in Table I. The average amount of total protein was 7·3 gm. per 100 c.c. of serum, of albumin 4·8 gm., and of globulin 2·5 gm. The average ratio of globulin to albumin was 0·52. This

TABLE I.
Blood proteins in normal specimens of S. rhesus.

Monkey.	Albumin (per cent).	Globulin (per cent).	Total protein (per cent).	Globulin Albumin
A ..	4·29	2·62	6·91	0·61
B ..	4·63	2·36	6·99	0·51
C ..	4·40	2·27	6·67	0·52
D ..	4·90	2·54	7·44	0·51
E ..	5·51	2·76	8·27	0·50
F ..	4·50	2·82	7·32	0·63
G ..	5·00	2·54	8·44	0·43
H ..	4·52	2·18	6·70	0·48
Mean ..	4·83	2·51	7·34	0·52

ratio was found to vary between the limits of 0.43 and 0.63 in different monkeys.*

(2) MONKEYS INFECTED WITH *PLASMODIUM KNOWLESI*.

(a) PRIMARY ACUTE INFECTIONS.

After having estimated the average protein figures in normal animals, some experiments were undertaken to ascertain the extent of any alterations which occurred in the blood of monkeys suffering from primary acute infections. The parasite used was *P. knowlesi*, and the infections were transmitted by blood inoculation. This parasite has a quotidian periodicity, and was specially chosen because it produces a very acute infection when injected into a susceptible species of monkey, such as *S. rhesus*. Such infections, if untreated, almost invariably prove fatal (*vide* Sinton and Mulligan, 1933).

The results of estimations of the blood proteins of the sera of these animals at various stages of the infection are given in Table II and Graph I. These showed that, when the parasitic attack became severe, there was a marked fall in the percentage of albumin with a slight rise in globulin. These changes were accompanied by a fall in the total proteins, and a rise in the globulin/albumin ratio.

The changes were most marked in Monkeys Nos. 556 and 558, which had acute, untreated, fatal infections. It is interesting to note that, in these two animals, the mere occurrence of detectable parasites in the peripheral blood, appeared to have little or no influence upon the blood proteins. It was only when the parasites had become very numerous that the change was noted. This was possibly due to a lag in the response to infection.

In Monkey No. 170, which was treated during the acute attack, the fall in total proteins was relatively less marked, due to the large increase in globulin. The globulin/albumin ratio was high in consequence.

In Monkey No. 145, which was first examined in the relatively quiescent interval after the primary acute attack, the total proteins and the albumin were below normal, while the globulin value was raised and the globulin/albumin ratio very high. This condition was probably due to the after-effects of the primary acute attack, during which the animal received treatment with quinine and plasmoquine on the 10th, 11th, 12th and 16th days to save its life. As the infection declined there was a distinct fall in the globulin/albumin ratio and in the percentage of globulin; the albumin rose slightly, *i.e.*, a tendency of the proteins to return to normal. The relatively quiescent interval was quickly followed by an acute recurrence of the infection which, being untreated, terminated fatally. In this attack the blood proteins behaved in a similar manner to that seen in the primary acute attack, except that the total proteins showed relatively little change, probably because they were already low as the result of the previous infection.

Summary.—When normal specimens of *S. rhesus* were infected with *P. knowlesi*, no changes were detected in the serum proteins until the parasitic infection became marked. The acute attack was characterised by a sharp

* For ease of comparison with the results obtained from the sera of infected animals, these limits and means have been indicated on the right-hand margins of Graphs I to VI.

TABLE II.

Blood proteins in acute infections with P. knowlesi in S. rhesus (vide Graph I).

Monkey num-ber.	Day of infec-tion.*	RESULTS OF PROTEIN ESTIMATIONS.				REMARKS.
		Albumin (per cent).	Globulin (per cent).	Total protein (per cent).	Globulin Albumin	
556	1	4.90	2.54	7.44	0.51	Before inoculation.
	2	4.88	2.50	7.38	0.51	No parasites seen.
	5	4.91	2.53	7.44	0.52	Very few parasites.
	9	4.10	2.83	6.93	0.70	About 50 per cent of blood cells infected.
558	1	5.51	2.76	8.27	0.50	Before inoculation.
	4	5.56	2.80	8.36	0.50	No parasites seen.
	6	5.53	2.80	8.33	0.51	Few parasites.
	8	4.30	3.06	7.36	0.71	More than 50 per cent of blood cells infected.
170	3	4.50	2.26	6.76	0.50	No parasites seen.
	13	3.67	2.97	6.64	0.81	Many parasites; treatment given.
145	27	3.05	3.30	6.35	1.08	Parasites few: recovering from attack.
	34	3.27	2.80	6.07	0.85	Parasites increasing after interval.
	39	3.00	3.10	6.10	1.03	Prior to death from very acute infection.

*In these and the later Tables, the date of inoculation is calculated as the first day of infection.

fall in the total protein and albumin percentages, accompanied by a slight rise in globulin. These changes resulted in a marked increase in the globulin/albumin ratio.

In Monkey No. 145, examined between the acute primary attack (treated) and the first acute recurrence, there was a tendency for the albumin and the globulin to return towards the normal limits, but the total proteins still remained low, while the globulin/albumin ratio was high. When the acute recurrence developed, changes in the albumin and globulin similar to those in the primary attack were noted, but to a less degree, probably because these fractions were already abnormal.

(b) CHRONIC INFECTIONS.

The results observed by us in acute primary attacks and in an early relapse have been very constant. It appeared possible, however, that different changes might be found during chronic infections. The conditions in such infections would be more comparable to those seen by many other workers

who studied chronic human infections. It was also necessary to determine whether, with the development of tolerance following upon prolonged chronic or latent infection, the changes disappeared or were modified. Fortunately we had available a large number of animals suffering from chronic infections, of which the histories were known in detail from continued blood examinations, usually daily, since the time of the first inoculation.

These animals could be divided into two classes—(i) those which had received multiple superinfections with heterologous strains of *P. knowlesi*, and (ii) those in which the multiple superinfections had been with homologous strains only (*vide* Mulligan and Sinton, 1933a, 1933b).

(i) *Chronic heterologous superinfections.*

Five animals were studied in this group, and the results are shown in Table III, and in Graphs II and III. As the result of prolonged chronic or latent infection and repeated superinfections, these animals had all developed a high degree of tolerance to superinfection with homologous strains of *P. knowlesi*, and a varying degree to the inoculation of heterologous strains (Mulligan and Sinton, 1933a, 1933b).

An examination of Graphs II and III shows that, in these five chronic infections (Monkeys Nos. 9, 80, 7, 29 and 39), the percentage of total proteins lay within normal limits in every instance, except in Monkey No. 7. On the other hand, there were definite changes in the proportion of albumin and globulin.

On two examinations in each of Monkeys Nos. 29 and 39, although the albumin percentage was just within the limits of normality, it was below the mean. In all the other estimations this percentage was distinctly subnormal. On the contrary, except for the first estimation in Monkey No. 7, all the globulin percentages were distinctly above the normal limits, and in many instances markedly so. Except in the first estimation in Monkey No. 7, the globulin/albumin ratio was high, sometimes being 1 or more.

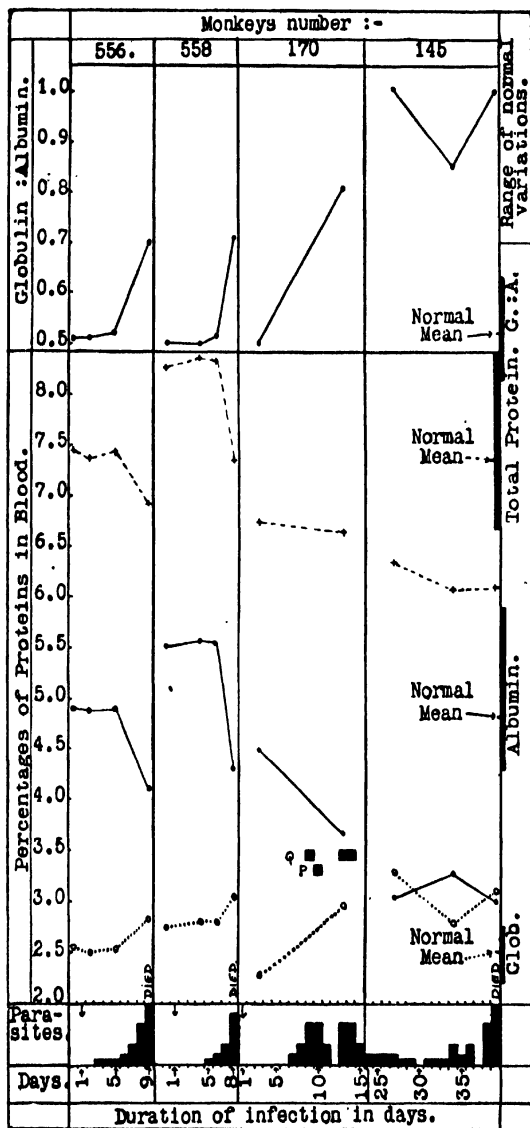
It is necessary to consider the histories of these animals during the period of observation, before a clear picture of the course of events can be obtained.

When the first estimation was made of the blood of Monkey No. 9, the animal was recovering from a parasitic relapse of moderate severity, following upon a heterologous superinfection with strain K₄. At this examination the total protein percentage was within normal limits, but the albumin was low and both the globulin percentage and the globulin/albumin coefficient were high. This state of affairs continued with little change up to the time of superinfection with the heterologous strain K₆. This superinfection terminated fatally in spite of treatment.* A blood analysis made on the day of death showed a drop in all the protein elements, and no change in the globulin/albumin ratio. As will be discussed later, the drop in the globulin percentage during an acute attack was possibly due to the effects of treatment.

Monkey No. 80 had shown but a slight parasitic infection for 10 days prior to the time when the first estimation was made. Although the total

* In the Graphs, treatment with quinine is indicated by the letter 'Q', with plasmoquine by 'P', with atebirin by 'A', and with stovarsol by 'S'.

GRAPH I.



GRAPH II.

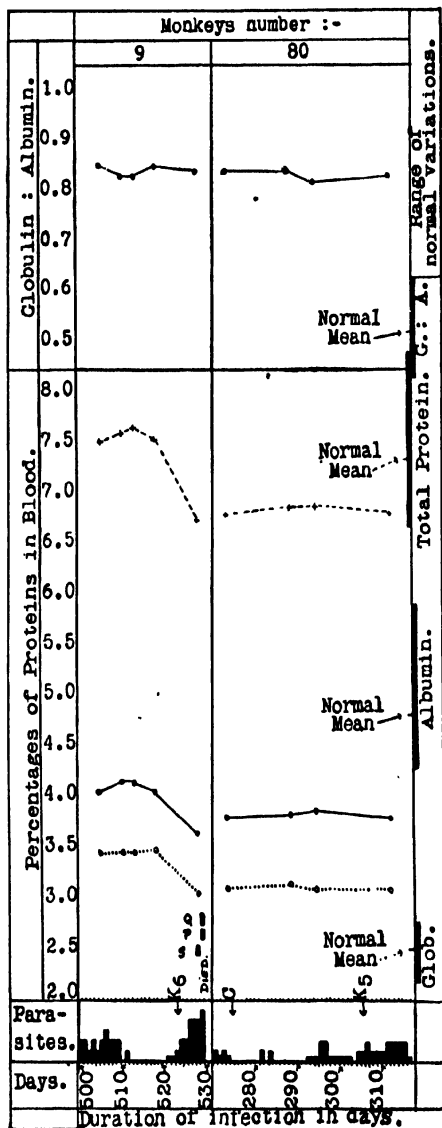


TABLE III.

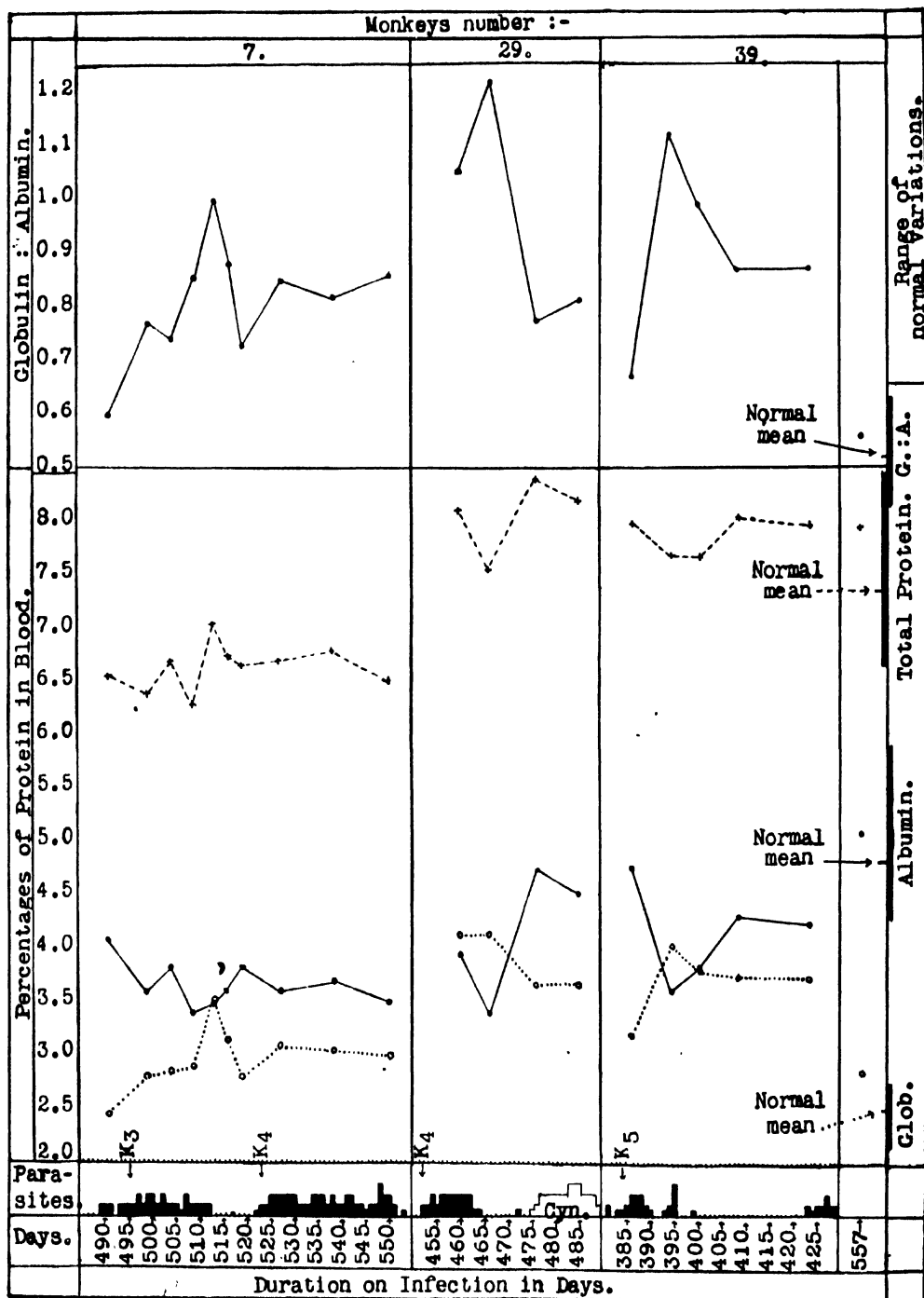
Blood proteins in chronic heterologous superinfections with P. knowlesi in S. rhesus (vide Graphs II and III).

Monkey num- ber.	INFECTIONS AND DAY.		Day of infection.†	RESULTS OF PROTEIN ESTIMATIONS.			Globulin Albumin.	REMARKS.
	Day.	Strain.*		Albumin (per cent).	Globulin (per cent).	Total protein (per cent).		
9	1	C	505	4.06	3.45	7.51	0.85	See details of parasites in Graph II.
	343	K ₁	510	4.16	3.45	7.61	0.83	
	379	K ₂	513	4.18	3.47	7.65	0.83	
	427	K ₃	518	4.07	3.49	7.56	0.85	
	484	K ₄	528	3.66	3.10	6.76	0.84	
	523	K ₅						
80	1	K ₈	273	3.70	3.10	6.80	0.84	See details of parasites in Graph II.
	126	Cyn	288	3.73	3.14	6.87	0.84	
	207	K ₁	294	3.78	3.10	6.88	0.82	
	274	C	312	3.72	3.10	6.82	0.83	
	305	K ₆						
7			491	4.10	2.45	6.55	0.60	See details of parasites in Graph III.
			499	3.61	2.80	6.41	0.77	
			504	3.84	2.86	6.70	0.74	
	1	C	509	3.39	2.92	6.31	0.86	
	343	C	513	3.50	3.52	7.02	1.00	
	379	K ₁	516	3.62	3.14	6.76	0.88	
	427	K ₂	519	3.84	2.80	6.64	0.73	
	494	K ₃	527	3.61	3.10	6.71	0.85	
	523	K ₄	538	3.76	3.05	6.81	0.82	
			550	3.50	3.01	6.51	0.86	
29	1	C	460	3.95	4.15	8.10	1.05	See details of parasites in Graph III.
	258	C	465	3.39	4.15	7.54	1.22	
	309	K ₁	476	4.74	3.67	8.41	0.77	
	357	K ₂	485	4.52	3.67	8.19	0.81	
	423	K ₃						
	452	K ₄						
39	1	C	386	4.77	3.21	7.98	0.67	See details of parasites in Graph III.
	203	K ₂	395	3.61	4.06	7.67	1.12	
	287	K ₁	400	3.84	3.80	7.64	0.99	
	343	K ₄	408	4.29	3.75	8.04	0.87	
			423	4.25	3.73	7.98	0.87	
			557	5.09	2.84	7.93	0.56	
	384	K ₇						

* This refers to the different kinds of strains of *P. knowlesi* reported by Mulligan and Sinton (1933b).

† The day of inoculation is counted as the first day of infection.

GRAPH III.



protein percentage was within normal limits, the albumin was low, and the globulin and the globulin/albumin ratio were both high. A heterologous superinfection with strain C caused little change in the infection or in the blood findings. The slight transient attack caused by a later heterologous superinfection with strain K₅ also gave rise to no apparent change in the blood proteins during the period of observation. It is possible that an examination made a few days later might have shown the usual alterations of proteins seen in an acute attack.

Monkey No. 7 had shown few or no parasites for about 10 days prior to the first protein estimation, nor had it had any severe parasitic attack for about 7 weeks. The findings suggest that during this quiescent interval the blood proteins had returned almost to normality. During a parasitic attack produced by a heterologous superinfection with strain K₅, the changes were similar to those found during other acute primary attacks. During the quiescent interval following this attack the proteins tended to return to their former level. When the animal was again superinfected with another heterologous strain (K₁), the usual protein changes occurred but to a less marked degree than in the previous attack.

The blood of Monkey No. 29 was first analysed about the middle of an acute parasitic attack following upon a heterologous superinfection with strain K₁. At this examination, although the total proteins were within normal limits, the albumin percentage was so low and the globulin so high that the ratio exceeded unity. Another examination made at the end of this attack showed an exaggeration of these changes. In the parasite-free interval which followed, the proteins showed a tendency to return rapidly towards normality, and the occurrence of a relapse due to *P. cynomolgi* produced but slight alteration.

The first estimation of the proteins of the blood of Monkey No. 39 was made a few days after a heterologous superinfection with strain K₅. Previous to this, parasites had been absent at nearly every daily examination for 3 weeks. The findings at this estimation showed the total protein and the albumin percentages to be within normal limits, but the globulin percentage and the globulin/albumin ratio were slightly higher than normal. The acute attack which followed this superinfection produced the usual blood-protein changes. These tended to disappear in the quiescent interval which followed the attack. A later estimation made on the 557th day, after an interval of about 6 weeks during which no parasites could be detected in the peripheral blood, showed that the blood proteins had returned to normal limits, except that the globulin was still slightly high.

Summary.—(i) Parasitic relapses, produced by heterologous superinfections during the course of chronic infections with *P. knowlesi*, caused protein changes similar to those seen in acute primary attacks, viz., (a) a fall in the total proteins, (b) a decrease in the albumin percentage, (c) a rise in the globulin percentage, and (d) an increase in the globulin/albumin ratio.

(ii) In the interval between attacks these protein elements tend to return to normal limits. However, there appears to be a definite tendency for the rise in the globulin percentage to persist for a longer period than the fall in total proteins and albumin.

(ii) Chronic homologous superinfections.

Three monkeys (Nos. 66, 64 and 141) were studied during the stage of tolerance following upon multiple homologous superinfections. The results of the blood-protein estimations made in these animals are shown in Table IV and Graph IV. From these it will be seen that the changes, both in the parasitic attacks and in the quiescent intervals, were very similar to those found in the case of heterologous superinfections. Here again the tendency was for the globulin percentage and the globulin/albumin ratio to remain above normal for long periods, while the albumin was usually low.

TABLE IV.

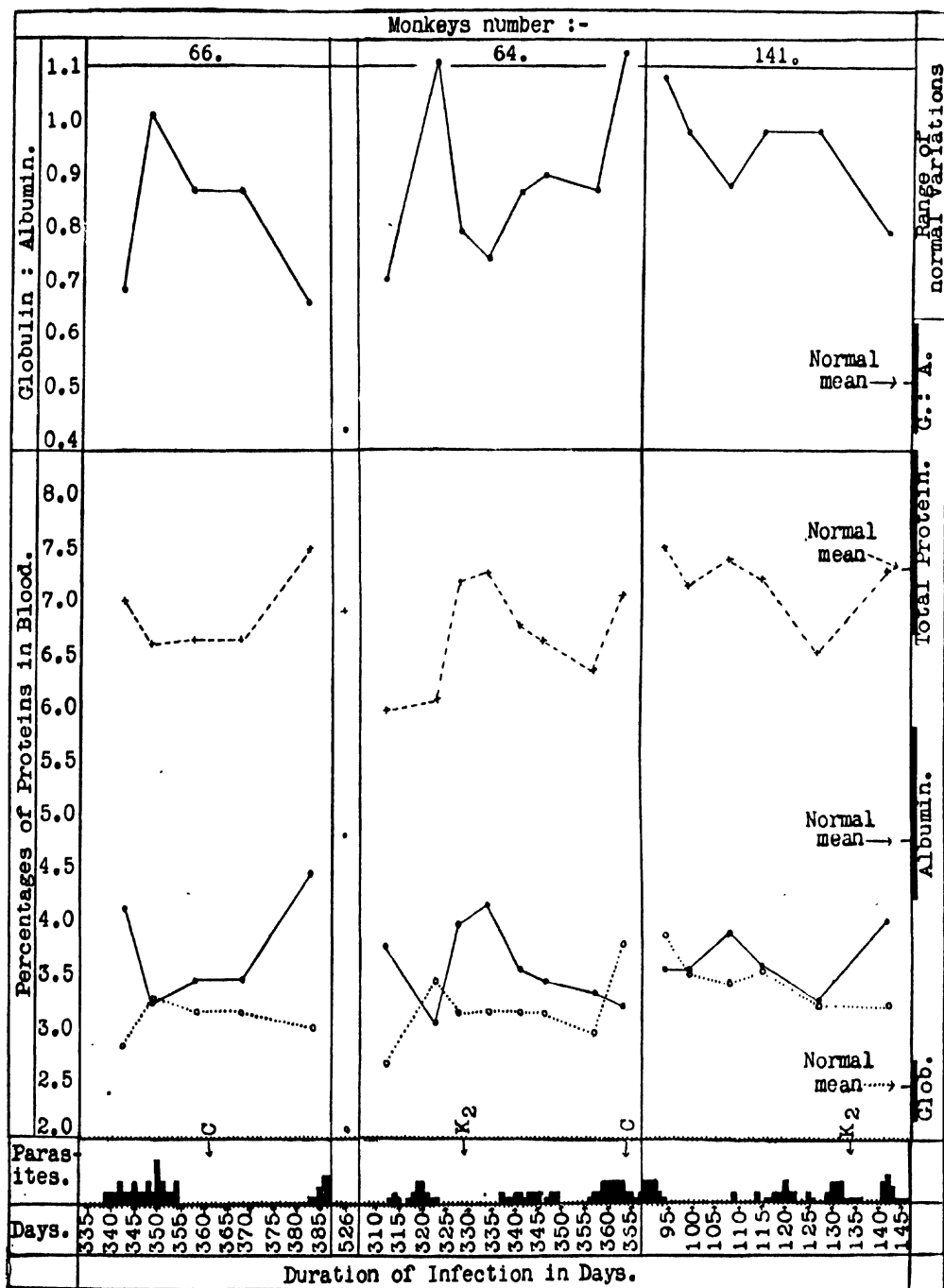
Blood proteins in chronic homologous superinfections with P. knowlesi in S. rhesus (vide Graph IV).

Monkey num- ber.	INFECTIONS AND DAY.		Day of infect- ion.†	RESULTS OF PROTEIN ESTIMATIONS.			Globulin Albumin.	REMARKS.
	Day.	Strain.*		Albumin (per cent).	Globulin (per cent).	Total protein (per cent).		
66	1	K ₁	342	4.16	2.89	7.05	0.69	See details of para- sites in Graph IV.
	209	K ₁	349	3.27	3.36	6.63	1.02	
	272	K ₁	358	3.56	3.19	6.69	0.88	
	292	K ₁	368	3.50	3.19	6.69	0.88	
	326	K ₁	383	4.51	3.05	7.56	0.67	
	361	C	526	4.86	2.10	6.96	0.43	
64	1	K ₂	312	3.80	2.71	6.51	0.71	See details of parasites in Graph IV.
	212	K ₂	323	3.11	3.49	6.60	1.12	
	251	K ₂	328	4.00	3.20	7.20	0.80	
	295	K ₂	334	4.19	3.19	7.38	0.76	
	329	K ₂	341	3.61	3.19	6.80	0.88	
	364	K ₂	346	3.50	3.20	6.70	0.91	
		C	357	3.39	3.01	6.40	0.88	
			363	3.27	3.84	7.11	1.14	
141			94	3.61	3.93	7.54	1.09	See details of parasites in Graph IV.
	1	K ₂	99	3.61	3.58	7.19	0.99	
	65	K ₂	108	3.95	3.49	7.44	0.89	
	134	K ₂	115	3.64	3.60	7.24	0.99	
			127	3.29	3.27	6.56	0.99	
			142	4.07	3.27	7.34	0.80	

* This refers to the different kinds of strains of *P. knowlesi* reported by Mulligan and Sinton (1933b).

† The day of inoculation is counted as the first day of infection.

GRAPH IV.



Monkey No. 100 (*vide* Table VII and Graph VI) was also examined once before splenectomy was performed during a chronic infection. No parasites had been found in the blood of this animal for about 7 weeks prior to the first protein estimation. Although the infection was a simple one of strain C, it showed the same peculiarities seen in multiple superinfections after a long quiescent interval.

Summary.—The blood-protein changes observed in monkeys with chronic infections with homologous strains were similar to those seen with heterologous ones, both in parasitic attacks and in quiescent intervals.

(iii) *Miscellaneous observations.*

The observations made on Monkeys Nos. 100 and 110 show some interesting features (*vide* Table VII and Graph VI).

(a) Monkey No. 100 had a simple infection with strain C, which had become very chronic. The animal had shown no parasites in the peripheral blood for about 7 weeks, after which it was splenectomised. An estimation of the blood proteins, made immediately before the operation, showed the globulin percentage and the globulin/albumin ratio to be raised, while the other figures were within normal limits.

Another estimation made 6 days after splenectomy, on the 2nd day of an acute parasitic relapse, showed a drop in all the proteins and also in the globulin/albumin ratio. These falls were most marked in the case of the globulin fraction, which came down to normal limits, and did not rise as is usual in an acute attack. This suggests that the increase of globulin in malarial infections may be connected intimately with splenic activity. This idea will be discussed later.

(b) Monkey No. 110 had been infected and superinfected with strain K₄. This animal was again superinfected with 1 c.c. of heart blood taken from Monkey No. 9 (*vide* Graph II and Table III) immediately after death. The latter animal had received superinfections with strains C, K₁, K₂, K₃ and K₄, and died in an acute attack following upon a superinfection with strain K₆. The dosage of parasites given was very large, more especially as the sample showed an extremely heavy infection, such as is seen in pernicious attacks at the time of death. The result of this large dose was that numerous parasites could be detected in the peripheral blood of the recipient animal on the same day, although none had been detected previously for 13 days, and only in very scanty numbers during the previous 6 weeks.

A blood estimation made on the following day, when the parasites were very numerous, showed the typical protein changes of an acute attack of monkey malaria. Unfortunately no estimations had been made prior to this inoculation, so one could not measure the relative degree of alteration. However, as this animal had previously been undergoing a prolonged quiescent interval, it is probable that the protein fractions were in the neighbourhood of normality, as had been found in other chronic infections of this nature (*vide supra*). It seems possible, therefore, that there was a very sudden change in the percentages of proteins in the blood, and that these were more rapidly developed than those observed after inoculation with smaller amounts of blood and with one strain only. Such a sudden change may be explicable as due to the large dosage of parasites of multiple strains, or possibly the effect of

protein shock upon a previously sensitised and hypertrophied spleen. The elucidation of the problem would, however, require further experimentation.

(3) MONKEYS INFECTED WITH *PLASMODIUM CYNOMOLGI*.

The bloods of five monkeys suffering from chronic infections with *P. cynomolgi* were studied, and the results are shown in Table V and Graph V. In addition to these, the blood of Monkey No. 29 was examined during a relapse due to this parasite in the course of a chronic infection with *P. knowlesi* (*vide* Table III and Graph III).

From these results it would appear that acute parasitic relapses due to *P. cynomolgi* cause changes in the blood proteins resembling those seen with *P. knowlesi*. The range of alteration was, however, comparatively slight, and the proteins tended to return rapidly to normality.

In the intervals between relapses of these chronic infections, the blood proteins remained within normal limits, and did not show the disturbed equilibrium seen in *P. knowlesi* infections.

On account of its tertian cycle of periodicity, the rate of multiplication of *P. cynomolgi* is slower than that of *P. knowlesi*, and the clinical manifestations of infection produced on inoculation into *S. rhesus* are of a much milder nature. Under these conditions it is not surprising that the changes produced should be less marked than with the severe infections produced by *P. knowlesi*.

As will be discussed later, these results bear a closer resemblance to those recorded by some workers in human cases, than do the results obtained with *P. knowlesi*.

(4) MONKEY INFECTED WITH *PLASMODIUM INUI*.

Only one animal was examined during a parasitic attack of this quartan infection. In the solitary estimation made, the blood proteins were found to vary within normal limits (*vide* Monkey No. 557 in Table V and Graph V).

TABLE V.

Blood proteins in infections with P. cynomolgi and with P. inui in S. rhesus (vide Graph V).

Monkey num- ber.	Parasite species.	Day of infection.	RESULTS OF PROTEIN ESTIMATIONS.			Globulin Albumin	REMARKS.
			Albumin (per cent).	Globulin (per cent).	Total protein (per cent).		
138	<i>cyn.</i> ..	94	4.80	2.36	7.16	0.49	Chronic infection.
		102	4.61	2.38	6.99	0.52	
		111	4.00	2.42	6.42	0.60	
137	<i>cyn.</i> ..	151	5.76	2.44	8.20	0.42	Chronic infection.
		165	5.63	2.44	8.07	0.43	
		176	5.31	2.58	7.89	0.48	
140	<i>cyn.</i> ..	58	4.63	3.10	7.73	0.67	Chronic infection.
		65	4.85	2.84	7.69	0.59	
		72	4.85	3.01	7.86	0.62	
		104	5.08	2.49	7.57	0.49	
		108	4.85	2.53	7.38	0.52	

TABLE V—concl'd.

Monkey num-ber.	Parasite species.	Day of infection.	RESULTS OF PROTEIN ESTIMATIONS.			$\frac{\text{Globulin}}{\text{Albumin}}$	REMARKS.
			Albumin (per cent).	Globulin (per cent).	Total protein (per cent).		
142	<i>cyn.</i> ..	128	4.97	2.40	7.37	0.48	Chronic infection.
		135	4.85	2.53	7.38	0.52	
		140	4.74	2.32	7.06	0.49	
		142	4.97	3.01	7.98	0.60	
152	<i>cyn.</i> ..	86	4.97	2.10	7.07	0.42	Chronic infection.
		100	5.10	2.32	7.42	0.45	
		119	5.31	2.40	7.71	0.45	
557	<i>inui</i> ..	22	4.74	2.44	7.18	0.53	Acute infection.

(5) SUMMARY OF THE RESULTS OF ESTIMATIONS OF THE BLOOD PROTEINS IN MONKEY MALARIA.

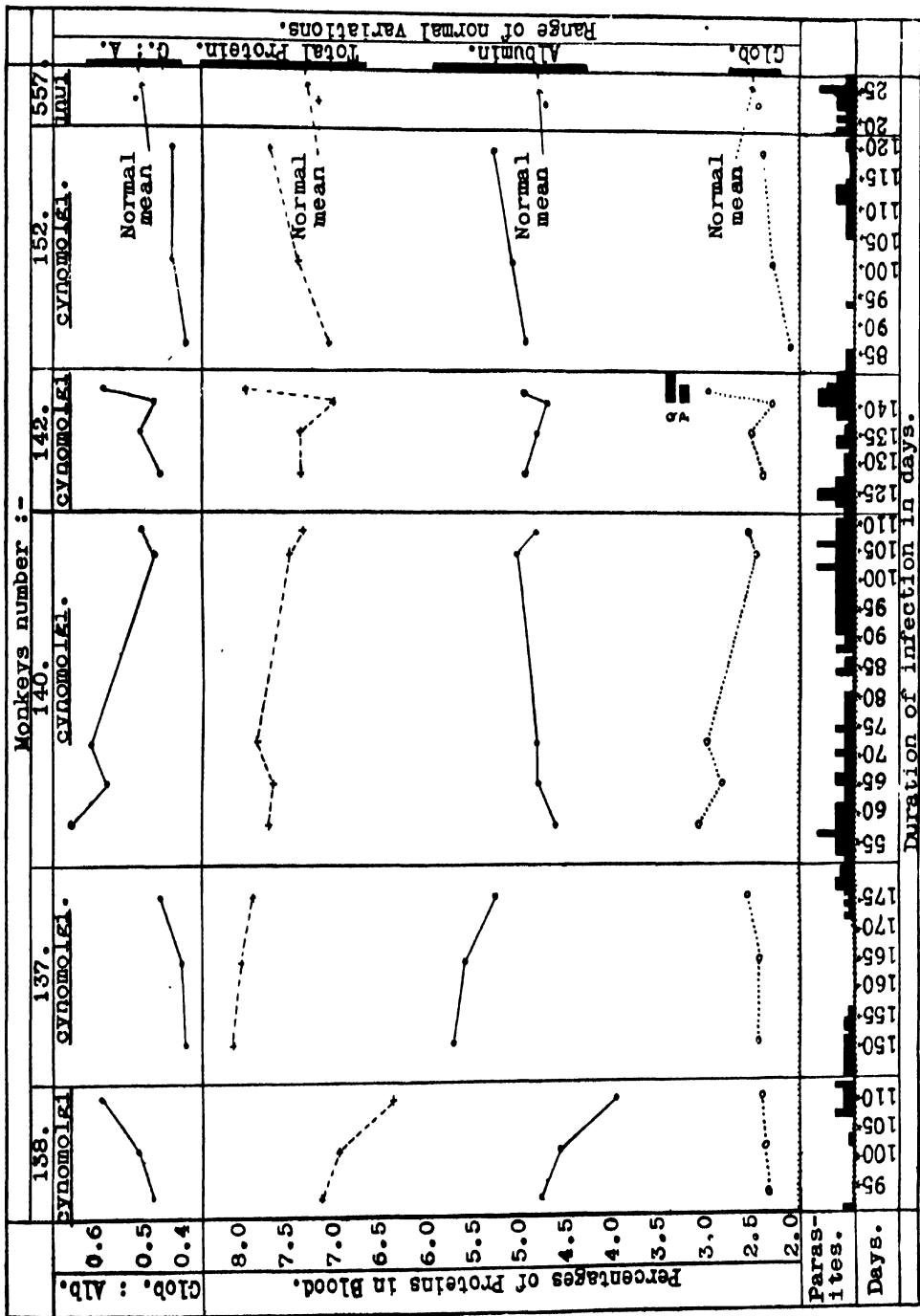
The results of our estimations of the serum proteins may be divided into those made on infections with *P. knowlesi* and those with *P. cynomolgi*. The individual observations have been recorded in Tables I to V, and these have been averaged in Table VI. In making an average of the estimations in acute infections, results obtained in these cases before the appearance of parasites in the peripheral blood, and in all cases, estimations likely to be influenced by treatment, have been excluded. It was hoped in this way to obtain a truer picture of the changes which occur in natural infections.

TABLE VI.

Average of serum-protein estimations in monkeys.

Type of infection.	Number of cases.	Number of estimations.	Albumin (per cent).	Globulin (per cent).	Total protein (per cent)	$\frac{\text{Globulin}}{\text{Albumin}}$
(a) <i>P. knowlesi</i> .						
(i) Acute ..	3	7	4.02	2.92	6.94	0.72
(ii) Chronic ..	8	47	3.85	3.20	7.14	0.85
(b) <i>P. cynomolgi</i> .						
Chronic ..	5	17	4.96	2.51	7.47	0.50
(c) Normal animals	8	8	4.83	2.51	7.34	0.52

GRAPH V.



These findings indicate that, in both acute and chronic infections of *P. knowlesi*, there is a fall in the amounts of total proteins and of albumin, with an increase in globulin and in the globulin/albumin ratio.

In the chronic infections, however, the decrease in albumin, and the increase in globulin are greater than in acute cases, which is probably due to a summation of effects. This results in a smaller fall in total proteins, and a greater rise in the globulin/albumin ratio in the former cases.

As noted above there is a great tendency for the protein equilibrium to return towards normal during the quiescent intervals of the infection. However, when a fresh parasitic relapse occurs the equilibrium is again disturbed.

In chronic infections of *P. cynomolgi*, the disturbances of the proteins are on the average slight, as compared with *P. knowlesi*. If the parasitic attacks in the individual cases be examined, it will be seen that variations in the proteins are of a similar type to, although smaller than, those seen in the latter infections.

The proteins in the one infection of *P. inui* examined were within normal limits.

(6) DISCUSSION OF THE CHANGES IN MONKEY MALARIA AS COMPARED WITH THOSE RECORDED IN HUMAN CASES.

Lloyd and Paul (1929) consider that the extent of the albumin reduction in malaria appears to have some connection with the intensity of the infection, and is not a function of the actual temperature. Tareev and Gontaewa (1933) also think that the fall in the albumin/globulin ratio is proportionate to the severity of the attack in natural malarial infections. In inoculation malaria they report that the latter ratio falls progressively with the number of rigors experienced by the patient.

If one examines the initial readings in the graphs given by Lloyd and Paul (1929), it will be found that in the case of benign tertian malaria the amount of albumin was, on an average, greater and the globulin less than in malignant tertian cases. This resulted in a higher globulin/albumin ratio in the latter cases.

A study of the protocols of the *knowlesi* infections in our series indicates that as the intensity of the malarial attack increased, as judged by the parasitic prevalence, so did the changes in the protein equilibrium. It is also seen that the more severe infection, *P. knowlesi* with its quotidian periodicity, caused much more marked changes than did the milder tertian one due to *P. cynomolgi*, or the quartan one due to *P. inui*. This appears to be similar to the reports on infections of *P. falciparum* as compared with *P. vivax*.

It may be that the changes found by us in infections with *P. knowlesi* are more marked than those recorded in human malarial infections, because of (a) the severity of the infection in non-immune animals, (b) the quotidian multiplication of the parasites, with no interval, not even a day, for recuperation, causing a greater summation of effects than in human malaria, and (c) of the fact that the majority of our cases received no treatment for the attacks being studied.

It is also to be noted that, with the appearance of the parasitic attack, the changes in the protein equilibrium did not become marked until the attack was well developed.

The fall in the serum proteins and albumin, with the rise in the globulin/albumin ratio, has been recorded by all the observers who have studied these features of the serum in human malaria. It is in relation to the globulin fraction that there is some absence of agreement between the different observers.

A rise in the serum globulin has been recorded in many bacterial infections and intoxications, both natural and experimental. Among protozoal diseases, Lloyd and Paul (1928) and Lloyd, Napier and Paul (1929) record a marked increase in the total serum globulin in developed and untreated cases of kala-azar. It is, therefore, not surprising that we should find an increase in the amount of this protein in malarial infections in monkeys.

Ogden (1930) reports an apparent increase in globulin in inoculation malaria. Lloyd and Paul (1929) show a slight rise in a case of malignant tertian malaria which had a relapse during the period of post-therapeutic observation. Pordes and Nguyen-Van-Lieng (1931), most of whose observations were made in chronic infections with *P. falciparum*, report an increase in globulin.

On the other hand, Dumolard *et al.* (1930) record a diminution of globulin in the chronic infections studied by them. Lloyd, Napier and Paul (1929) and Lloyd and Paul (1929) found that, in the relapses of chronic infections investigated by them, as a rule the globulin was low, but in some cases the decrease was slight or absent. Tareev and Gontacwa (1933) state that, in inoculation malaria due to *P. vivax*, the globulin was lowered slightly, if at all. James (1933) says that there is some reduction in the amount of total globulin.

As noted above, the degree of protein disturbance appears to vary with the intensity of the infection, so the discrepancies in the different findings may depend upon this factor. It is seen from the above records that globulin appears to be increased more often in the severer malignant tertian infection than in the milder benign tertian. This would be in keeping with our findings in *P. knowlesi* infections as compared with *P. cynomolgi*. Although Lloyd and Paul (1929) studied some cases of infection with *P. falciparum*, it must be remembered that they made only one estimation in each case before treatment was started to control the parasitic attack. As noted above, it apparently takes some days for the protein changes to develop, so it is quite possible that, if treatment had been withheld and another estimation made, Lloyd and Paul would also have found a rise in the globulin fraction, as they did in the malignant tertian relapse noted above. The initial absence of a raised globulin value in these cases may also have been due to the fact that human patients usually receive some sort of treatment during their acute attacks. The disturbance of the protein equilibrium was therefore less likely to reach the height seen in the chronic infections of our untreated animals.

We have been unable to study the protocols of several of the observers who reported little or no rise in globulin. It is possible, however, that similar explanations might account for their failure to find any rise in total globulin.

Numerous workers have noted the relationship of increased serum globulin, especially the euglobulin portion, to certain serum reactions such as the formol-gel, the novocaine-formalin, etc., and recent work would appear to indicate that Henry's reaction in malaria is not a specific antigen-antibody reaction, as originally claimed, but is due to an increase in some portion of the globulin fraction in the serum of malarial cases.

As a result of our work and a study of that reported by other observers, it appears to us that, as found in other infections, the globulin tends to rise during an attack of malaria, and that the degree of the rise is probably influenced largely by the severity of the attack.

V. THE EFFECTS OF TREATMENT UPON THE DISTURBED EQUILIBRIUM OF THE BLOOD PROTEINS IN MALARIA.

During the course of these experiments a number of the animals received treatment with different anti-malarial drugs. The drugs used were:—

- (i) Quinine sulphate in daily doses of 2 grains orally;
- (ii) Plasmoquine injected intramuscularly in doses of 0.005 gm. daily;
- (iii) Atebrin in daily doses of 0.025 gm. orally; and
- (iv) Stovarsol by the mouth in doses of 0.013 gm. daily.

The average weight of the animals in our experiments was about 2,400 gm.

Quinine and plasmoquine treatment were combined in some instances (*vide* Mulligan and Sinton, 1933a). Stovarsol was given rather as a general tonic, etc., than for its parasitocidal action, because this drug had proved in our experience to be very efficacious in the severe anæmias which sometimes follow an acute attack of monkey malaria.*

Various observations were made during the treatment of 7 different monkeys infected with *P. knowlesi*. The animals studied were—No. 170 (Table II, Graph I), No. 9 (Table III, Graph II), No. 66 (Table IV, Graph IV), and Nos. 203, 204, 100 and 110 (Table VII, Graph VI).†

(1) EFFECTS OF TREATMENT ON ACUTE PRIMARY ATTACKS.

In the severe primary attacks produced by the inoculation of *P. knowlesi* in *S. rhesus* (Nos. 203 and 204), the administration of atebrin appeared to check to a great extent the rise in the globulin/albumin ratio, and the fall in the total protein and albumin percentages. In comparison with untreated animals (*cf.* Nos. 556 and 558) the changes were much less marked. On the other hand, the globulin fraction did not rise but remained stationary or fell slightly. The treatment, therefore, seemed less effective in its control of the fall of albumin than in the rise of globulin.

During the primary acute attack in Monkey No. 170, both quinine and plasmoquine were given to control the infection. Unfortunately the number of observations on this animal were too few from which to draw any conclusions,

* This method of treatment for the malarial anæmias seen in monkeys was previously advocated by Sinton and Mulligan (1933), and continued use has confirmed its value. The exact action of this organic arsenical preparation in such anæmias is uncertain. The effects produced frequently appeared to be too rapid to be due merely to a stimulation of the hæmopoietic system to produce more erythrocytes. It is possible that its action is upon the reticulo-endothelial system. In many of our monkeys dying from malaria, we have noted at autopsy a very marked phagocytosis of red cells by the macrophages of this system. It is possible, therefore, that the severe progressive anæmia, usually associated with a very low grade infection, may be related to an abnormal activity of such phagocytic action. Several workers have noted an affinity of organic arsenical preparations for the cells of the reticulo-endothelial system, so it may be that these drugs not only stimulate the hæmopoietic system to regenerate erythrocytes but also diminish the phagocytic action of certain macrophages upon red cells.

† In the Graphs, treatment with quinine is indicated by the letter 'Q', with plasmoquine by 'P', with atebrin by 'A', and with stovarsol by 'S'.

but it is seen that the fall in total proteins and in albumin seems less than in untreated cases in proportion to the severity of the attacks.

TABLE VII.

Blood proteins in infections treated with anti-malarial drugs (cf. Graph VI).

Monkey No.	Parasite species.	Day of infection.	RESULTS OF PROTEIN ESTIMATIONS.			Globulin Albumin	REMARKS.
			Albumin (per cent).	Globulin (per cent).	Total protein (per cent).		
203	<i>knowlesi</i>	5	4.87	2.24	7.11	0.46	Treated with atebrin on days:—9, 10, 11, 12 and 19.
		8	4.74	2.10	6.84	0.46	
		10	4.29	2.01	6.30	0.47	
		20	4.52	2.44	6.96	0.54	
		29	4.15	2.64	6.79	0.64	
204	<i>knowlesi</i>	5	5.65	2.48	8.13	0.44	Treated with atebrin on days:— 9, 10, 20, 21 and 22.
		8	5.31	2.36	7.67	0.44	
		12	4.52	2.40	6.92	0.53	
		20	4.20	2.66	6.86	0.64	
100	<i>knowlesi</i>	149	4.75	3.20	7.95	0.67	Treated with (a) quinine on days:— 156, 157, 158, 162, 163 and 164; and (b) plas- moquine on days:—156 and 157.
		155	4.50	2.66	7.16	0.59	
		158	4.63	2.66	7.29	0.58	
		164	4.63	2.62	7.25	0.57	
110	<i>knowlesi</i>	214	3.85	3.31	7.16	0.86	Treated with both quinine and plasmoquine on days:—213 and 214.
		221	5.08	2.85	7.93	0.56	
		223	5.02	2.90	7.92	0.58	

(2) EFFECTS OF TREATMENT ON ACUTE ATTACKS IN CHRONIC INFECTIONS.

In the case of Monkey No. 9, stovarsol was given during an acute parasitic attack following a heterologous superinfection. Although the attack terminated fatally, no change was found in the globulin/albumin ratio and the fall in albumin was relatively small. There was, however, a distinct drop in the globulin fraction, but whether this fall was due to the action of stovarsol is uncertain.

The blood was examined during the course of parasitic attacks occurring in chronic infections of *P. knowlesi* in Monkeys Nos. 100 and 110. As the former of these had been splenectomised shortly before, and the latter had received a massive blood inoculation with several heterologous strains at the same time, it is doubtful in how far these procedures influenced the blood proteins, irrespective of the effects of treatment.

Both these animals were treated with quinine and plasmoquine. In Monkey No. 100 there was a marked fall in the total protein and globulin percentages after splenectomy, and the acute attack which followed the operation, when treated with quinine, produced no striking changes in the protein elements of the serum. Unfortunately only one estimation was made of the serum proteins of Monkey No. 110 during treatment, so it is impossible to say what effect treatment had. All the protein fractions, however, showed a most striking return towards normality during the next week, which was probably due mainly to the result of treatment.

Monkey No. 66 received treatment with stovarsol during the course of an acute parasitic attack of *P. knowlesi* in a chronic infection. In this instance, the serum showed little change in the total proteins, but a slight rise in albumin and a fall in globulin. The latter changes resulted in a drop in the globulin/albumin ratio, and, after the termination of treatment, all the protein fractions tended to return rapidly towards the normal limits.

One estimation was made during the treatment of a chronic infection of *P. cynomolgi* with quinine and plasmoquine. At this observation a rise in all the protein fractions was recorded, more especially with the globulin. Whether another estimation a few days later would have revealed a fall, it is impossible to say.

Summary.—From the evidence available it would appear that the administration of anti-malarial drugs, such as quinine, atabrin, plasmoquine and stovarsol, tends to diminish the extent of the protein changes produced by acute untreated attacks of monkey malaria. Such treatment also seems to increase the rapidity with which any disturbance of the protein equilibrium disappears after an attack. The evidence suggests that treatment had a distinct effect in preventing the rise in globulin seen in untreated attacks, and may even cause a fall in this element.

(3) DISCUSSION OF THE EFFECTS OF TREATMENT.

The effects of treatment upon the disturbed serum-protein equilibrium in human malaria have been studied by Radosavljevic' and Ristic' (1926), by Lloyd and Paul (1929), and by Tareev and Gontaewa (1933). All these observers report that anti-malarial treatment assists the return of serum proteins to their normal values. This return is said to be rapid in most cases.

The work of Lloyd and Paul (1929) has been more detailed than that of other observers. They report that with treatment (a) there is a slight lag between the defervescence of fever and the return of the proteins towards normality; (b) the rise of the albumin curve is at first very rapid, but becomes less marked as normality is approached; (c) the raised globulin/albumin ratio falls with treatment; and (d) quinine treatment appears to have little effect upon the globulin value in the majority of instances, 'only in those cases where it is depressed initially does the globulin value rise under the influence of quinine'.

These findings, except for the globulin values, are very similar to our observations on monkey malaria, *viz.*, a fall in the globulin/albumin ratio, and a rise in the albumin and total protein percentages. In the case of the globulin fraction our results would appear to indicate a tendency for the amount of this protein to remain stationary, or to fall slightly with treatment if already

raised. The changes were, however, slight, and the result of treatment would appear to be an inhibition, temporarily at least, of any further deviation from normal of the globulin value during the attack.

It is interesting to note that in another protozoal disease, kala-azar, Lloyd, Napier and Paul (1929) found a raised value for globulin in untreated cases. They report that the effect of treatment in such cases was an immediate and tremendous fall in globulin (due mainly to a decrease in pseudoglobulin), and that a corresponding enormous rise in the albumin also occurred immediately. This suggests a similar change to that seen in some of our monkeys.

Whether the action of anti-malarial drugs, in causing a rapid return of the serum proteins towards their normal values, is due to the influence of these drugs in reducing the intensity of the parasitic infection, or from some effect upon the disturbed protein metabolism in malaria, or both, is impossible to determine definitely from the evidence available. It is, however, probably due mainly to the first cause.

Lloyd and Paul (1929) say that 'if further information should become available to point to the conclusion that the protein changes in the malarial relapse are evidence of the recommencement of the immunisation response, it will raise the question whether it is wise to terminate this "natural" response by the administration of quinine'. This suggestion is in line with that of James (1931) that the therapeutic action of quinine in benign tertian malaria is considerably enhanced, if the patient be allowed to develop a certain degree of tolerance to the infection before treatment commences.

VI. GENERAL DISCUSSION UPON THE CAUSATION OF THE DISTURBANCES IN THE EQUILIBRIUM OF THE SERUM PROTEINS IN MALARIAL INFECTIONS.

In any such discussion it is necessary to consider various points in relation to the origin of the blood proteins, and the factors influencing the amounts of these substances in the blood.

(1) THE ORIGIN OF THE BLOOD PROTEINS.

The work which has been done in attempts to solve this problem has been very fully reviewed by Reimann *et al.* (1934). From the information given in this review, it appears that the liver is probably the chief source of fibrinogen, if not the only site of its formation.

The other sources suggested for the blood proteins are (a) from the disintegration products of leucocytes, (b) from the products of the destruction of erythrocytes, (c) from the intravascular disintegration of platelets, (d) from the bone-marrow, lymphoid organs and spleen, (e) from the disintegration of cells generally, and (f) a humoral origin. Herzfeld and Klinger (1917) support the cellular origin of the blood proteins, and suggest that, after the disintegration of the cells, the resultant large molecules comprise the fibrinogen fraction. Further dispersion they think may give rise to a series of fractions composed of smaller particles—in turn euglobulin, albumin and finally incoagulable proteins.

After considering all the work on this subject, Reimann *et al.* (1934) decided that the source of origin of the blood proteins was still unsolved.

This review is specially interesting to the malariologist, because many of the suggested sources of the blood proteins are markedly affected by malarial infections. It is not surprising, therefore, that in this disease there occurs a definite change in the serum-protein equilibrium.

(2) CHANGES IN THE SERUM-PROTEIN EQUILIBRIUM.

A fall of the total serum proteins and of the albumin fraction, combined with a rise in the globulin/albumin ratio, are the chief features recorded by different observers who have studied this aspect of malarial infections.

Moschcowitz (1933), in a discussion of the serum proteins, points out that the rise in the globulin/albumin ratio is manifest in hypoproteinæmias of any origin. It cannot, therefore, be considered as specific for malaria. Moschcowitz (1933) states that the cause of this change is not clear, but 'one may conjecture that the reversal is due partly to the fact that in hypoproteinæmia it is albumin and not globulin that is lost, and partly to the power of the body to regenerate globulin in such conditions, as a compensatory measure'. This author found, as did several other workers, that hypoproteinæmia is fairly frequent in pernicious anæmia, and it is suggested that this may be due either to a deficient formation or to a destruction of plasma proteins. He also notes loss of blood as one of the causes of deficient proteins. These findings suggest that the anæmia and loss of blood in malaria, may have some relationship to the protein changes in this disease.

Hurwitz and Meyer (1916) and Hurwitz and Whipple (1917) report that, in the process of bacterial infection in animals, there is a change in the globulin/albumin ratio with an increase in globulin. These effects could be produced by the injection of either living or dead organisms, and by bacterial endotoxins. If massive doses of such material were given, the result was usually an upset of the globulin/albumin ratio which showed no tendency to right itself for a long time. When the infection was overwhelming, the tendency for the heaping up of the globulins was more likely to occur. This rise may be due in theory to a more rapid formation, or a less rapid destruction or utilisation.

Richetti (1916) found that the injection of typhoid vaccine into rabbits, previously non-immunised, caused an absolute decrease in albumin and an absolute increase in globulin. Re-immunisation by later doses of vaccine caused a further fall in albumin, but the globulin was unaffected. Lloyd and Paul (1929) tried the effects of the inoculation of a mixed typhoid-paratyphoid vaccine into human cases. The primary inoculation resulted in a sharp rise in serum globulin and a fall in albumin. After a second inoculation given 10 days later, no further change in globulin was detected but the albumin fell still lower.

The results obtained by bacterial infections, inoculations and intoxications appear to be very similar to those detected in malarial cases. The disturbances in protein equilibrium cannot, therefore, be considered as specific for malarial infections.

(3) CHANGES IN THE SERUM-PROTEIN EQUILIBRIUM IN RELATION TO THE RETICULO-ENDOTHELIAL SYSTEM.

That the reticulo-endothelial system plays a preponderant part in the disturbance of the protein equilibrium is suggested by Kisch (1923) and by Du Bois (1931).

Much research work has been carried out in recent years in attempts to elucidate the functions of the reticulo-endothelial system. Many of these researches have been based upon the changes produced when attempts were made to block this system in animals by means of various substances of a particulate nature. It is also believed that injections of various types of foreign protein tend to block the system. Repeated hæmorrhages have also been found to affect its function.

When one considers the close relationship between the reticulo-endothelial system and the effects of malarial infection, it seems likely that the blood protein variations found in this disease may be dependent largely upon changes in that system.

Berger (quoted by Du Bois, 1931) found that the parenteral injection of foreign protein into animals caused a disturbance of the equilibrium of the blood proteins, which was shown by a diminution in albumin and a rise in globulin, with an increase in the globulin/albumin ratio, appearing rapidly after injection. Derer and Steffanutti (1930) report similar results following the intravenous injection of histamine into rabbits and cats.

Du Bois (1931) found that the immediate effects of the intravenous injection of Indian ink, collargol, or 'soluprotein Roche', in attempts to block the reticulo-endothelial system, were a fall in albumin and a rise in globulin. Later there was a tendency for the total proteins to return to their normal value, but this total was made up of disproportionate amounts of albumin and globulin (*cf.* our results in monkey malaria). 'These results appear to me to confirm the view of the cellular origin of the variations in blood proteins; it seems plausible to admit that the reticulo-endothelial system plays a preponderant rôle in this phenomenon'.

Brandes (1934) also carried out experiments on animals, and concludes that stimulation of this system by various means, such as the injection of foreign proteins, or particulate matter, and repeated hæmorrhages, apparently produces an increase in the *plasma* proteins, particularly the globulin. The changes were marked after bleeding and the injection of graphite.

As pointed out by Klein and Levinson (1933) the effects on the blood caused by reticulo-endothelial blockage, induced by different substances, depend upon the species of animal used, the amount and nature of the material injected and the length of time after the injection that the observations are made. They consider that the most important factor influencing the result recorded is whether the reticulo-endothelial system has been stimulated or depressed by the experimental measures used.

If one considers the effects of a malarial infection upon the host, as compared with the experimental measures mentioned above, the resemblance is close. In malaria we have (a) a liberation of foreign particulate matter into the blood stream, when the segmenting parasites burst and set free the pigment granules, (b) at the same time the blood receives the remains of altered, parasitised erythrocytes, and the debris of the mature parasites left after the liberation of the merozoites, all of which may act as foreign proteins, and (c) there is also an enormous destruction of erythrocytes, which may cause results similar to those produced by repeated bleeding or severe anæmia.

Under these conditions it is not surprising to find that the serum proteins in malaria react in a manner suggesting that their disturbed equilibrium is closely connected with the effects of this disease upon the reticulo-endothelial system. Many of the points raised are, of course, largely speculative, but appear worthy of consideration in any more detailed examination of this problem.

VII. SUMMARY.

Under the conditions of our experiments we have found that—

(1) Acute parasitic attacks in simian malarial infections produce a disturbance in the serum-protein equilibrium characterised by (i) a fall in the total proteins, (ii) a decrease in the albumin, (iii) a rise in the globulin, and (iv), as a result, an increase in the globulin/albumin ratio.

(2) The degree of disturbance appears to be related directly to the intensity of the attack. The results of the more severe infection caused by *P. knowlesi* are more marked than those produced by *P. cynomolgi*.

(3) In the quiescent intervals between attacks, the proteins tend to return towards normality, until again disturbed by a parasitic relapse. Although the total proteins may come back to normal limits in the quiescent interval, the amount of globulin to albumin is frequently disproportionately large, as shown by an increased globulin/albumin ratio.

(4) The return of the blood proteins towards their normal values appears to be accelerated by the treatment of the attack with quinine, atabrin, plasmoquine or stovarsol.

(5) From an examination of the evidence available, it appears probable that the disturbance of the serum-protein equilibrium in malaria is caused, mainly if not entirely, by the effects of this disease upon the reticulo-endothelial system and probably is not specific to malaria.

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ATEBRIN MUSONATE : A NOTE ON THE RATE OF
ABSORPTION AND ON THE LOCAL EFFECTS
OF INTRAMUSCULAR INJECTION.

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THE drug usually known as atebtrin is the dihydrochloride of the base atebtrin. The makers have recently prepared the methyl sulphonic acid salt of the same base under the laboratory name of 'atebrin musonate', of which 0.125 gm. is equal in atebtrin content to 0.1 gm. of atebtrin dihydrochloride. It is sold as 'Atebrin for Injection', and must be distinguished from the older preparation known as 'Atebrin *tablets* for Injection'. The latter is the dihydrochloride. Atebrin musonate is a yellow powder, and is packed in ampoules labelled 0.1 and 0.3 gm. These figures represent the contents in terms of the more familiar atebtrin dihydrochloride. The actual weights of atebtrin musonate in the ampoules are 0.125 and 0.375 gm. respectively. In this paper, the word 'ampoule' refers to the smaller size.

The makers recommend that the contents of one small ampoule should be dissolved in 3 c.c. of water, and that the dose should be one ampoule for intravenous and one to three for intramuscular injection. It is not at present sold in a form intended for oral administration.

Atebrin musonate has been used successfully by Blaze and Simeons (1935) in Colombo. They stated that they could control the disease by one intramuscular dose of three ampoules on each of two successive days. Although

parasites might reappear in the blood, there was no return of fever during a period of observation apparently not exceeding seventeen days.

Atebrin dihydrochloride has been injected intramuscularly into man, monkey and other animals (Eckhardt, 1933, Hoops, 1933, Hecht, 1933, and Nauck, 1934). Eckhardt found that, after repeated injections, there was local infiltration, which quickly disappeared without leading to abscess formation. Hecht reported that it had a certain irritant action on the tissues, but it did not produce necrosis. Other authors observed no local reaction. According to Blaze and Simeons (1935), the injection of atebrin musonate 'proved to be painless; no local irritation or reaction was seen in any cases of this series'. Similarly many thousand doses of quinine have been given without obvious ill-effects at the site of injection. But those who have looked beneath the surface have seen that quinine always causes necrosis, and that the results are sometimes disastrous (MacGilchrist, 1917, Dudgeon, 1919, Figdor and Pinnock, 1922, Fletcher and Visuvalingam, 1923, and Karamchandani, 1933). It therefore seemed worth while to determine by observation in monkeys the nature of the local action of atebrin musonate, and at the same time to estimate the comparative rates of absorption, when the drug is given into a vein or muscle or by the mouth.

TECHNIQUE.

The monkeys used were *Silenus rhesus*, weighing between 1.80 and 4.55 kg. In every case, the dose per kilogramme was 5.36 mg. of atebrin musonate (4.29 mg. atebrin dihydrochloride). The equivalent dose in a man of 70 kg. is three ampoules of atebrin musonate or 0.3 gm. of atebrin dihydrochloride. Each monkey received one dose only. When the intramuscular route was used, the drug was injected deeply into the muscles on the outer and posterior aspect of the thigh, at a point midway between the knee-joint and the tuber ischii. Oral doses were given into the stomach through a rubber tube.

Immediately after the administration of the drug, the monkey was placed in a small cage. A funnel under the cage collected the urine into a bottle. At the end of six hours this bottle was removed, and the amount of atebrin in the urine was estimated by the method of Wats and Ghosh (1934). The amyl alcohol used for the extraction of the atebrin also extracted a certain amount of extraneous colour, which was perhaps derived from faecal contamination. This colour was removed by acidifying the urine, shaking with lead acetate (1 gm. to 100 c.c. of urine) and filtering, before proceeding to the estimation in the usual way. Treatment with lead acetate also had the advantage that it rendered unnecessary the final centrifuging of the amyl alcohol extract.

RESULTS.

LOCAL ACTION ON TISSUES.

Eight monkeys received intramuscular injections of atebrin musonate solutions of varying concentrations (see Table). The tissues at the site of inoculation were examined forty-eight hours later.

TABLE.

Excretion in the first six hours after the administration to monkeys of atebtrin musonate by different routes. The dose in each case was 5.36 mg. per kilogramme of body weight. The atebtrin excreted is calculated as atebtrin musonate.

Monkey number.	Weight in kg.	Route.	One ampoule dissolved in — c.c. water.*	Six hours' excretion in mg.	Percentage of dose excreted in six hours.
1	3.95	Muscular	4.5	0.17	0.8
2	3.10		3.0	0.20	1.2
3	4.20		3.0	0.32	1.4
4	2.00		3.0	0.09	0.9
5	4.35		1.5	0.14	0.6
6	1.50		1.5	0.21	2.2
7	3.50		1.0	0.11	0.6
8	1.85		1.0	0.13	1.3
9	1.90	Venous	3.0	0.07	0.7
10	1.80		3.0	0.20	2.1
11	2.20		3.0	0.11	0.9
12	1.95	Oral	3.0	0.07	0.7
13	3.15		25.0	0.02	0.1
14	2.40		25.0	0.11	0.8
15	4.55		25.0	0.04	0.2
16	1.85	"	25.0	0.06	0.6

*The strength of solution recommended by the makers is one ampoule dissolved in 3 c.c. of water.

Monkey no. 7 was the only one to show any reaction. In none of the others was there any damage to the tissues which was perceptible to the naked eye, nor was there any trace of yellow colour.

The solution injected into monkey no. 7 was made by dissolving one ampoule in 1 c.c. of water. This is three times the strength recommended by the makers. In this case, there was at the site of injection an area of 1.0×0.5 cm., in which there was congestion and hæmorrhage, with œdema of the muscle and adjoining connective tissues. No yellow colour was seen.

RATE OF ABSORPTION.

The table shows large variations in the percentages of the dose which were recovered from a six hours' specimen of urine. Such a specimen is not a strictly accurate measure of the amount excreted by the kidney, because there is no guarantee that the bladder is empty at the end of the six-hour period. Another source of error is introduced by the individual factor. The latter could have been evaded by giving the drug to each individual by the venous, muscular and oral routes in succession. But owing to the length of time during which a single dose of atebtrin remains in the body, it would have been necessary to allow several weeks to intervene between successive doses, and sufficient time was not available for this method.

Owing to these variations, it is not possible to establish a numerical relationship between the times of excretion when the drug is given by different

routes. However, it is probably fair to say that it was excreted more rapidly, and presumably absorbed into the general circulation more rapidly, when injected into a vein or muscle than when given by the mouth. But on the basis of these figures it would be unwise to distinguish either the venous or muscular routes as leading to more rapid absorption.

SUMMARY.

(1) Under the conditions of this experiment a single dose of atebrin musonate was found to have no injurious action on the tissues of monkeys when injected intramuscularly in the strength recommended by the makers.

(2) It appeared to be absorbed more quickly when injected into a vein or muscle than when given by the mouth.

ACKNOWLEDGMENT.

I am indebted to Dr. O. Urehs, of the Haverø Trading Company, for the information given in the first paragraph of this paper, and for supplying samples of 'Atebrin for Injection'.

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AN INSTANCE OF SPECIES ANOMALY AMONGST ANOPHELINES.

BY

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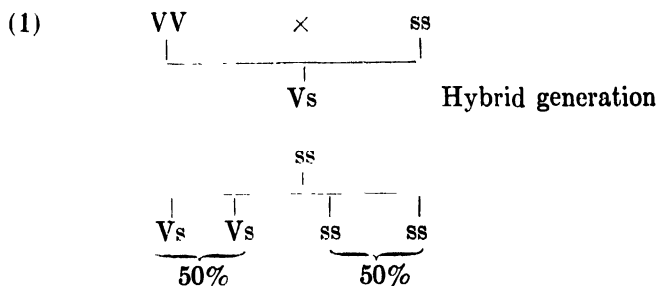
[27th May, 1935.]

RECENTLY while I was carrying on some breeding experiments with Anophelines, I came across a curious instance where a female *Anopheles vagus* produced larvæ of both *vagus* and *subpictus* (*rossi*) types, from which eventually mosquitoes of these two species emerged in equal proportions.

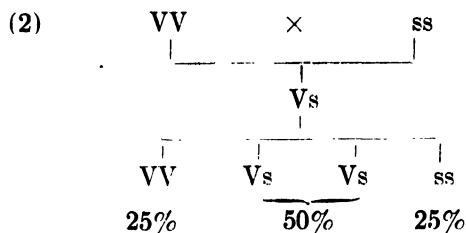
Such a combination is likely to be obtained in instances of hybridisation only. In the light of Mendelian principle, if by crossing a *vagus* with a *subpictus* specimen it was possible to get a hybrid generation with *vagus* characters dominating, and if there was a mating between this resultant hybrid *vagus* form with a pure *subpictus* form, then only we might get in the next generation 50 per cent *vagus* resembling the hybrid parent and 50 per cent *subpictus*, or *vice versa* if the hybrid generation had *subpictus* characters dominating. I therefore believe Mendelian segregation has occurred in the present case.

To be more explicit, suppose 'V' represents the factor which determines the character of a *vagus* form and 's' its absence, then the pure *vagus* type will be 'VV' and the *subpictus* type 'ss', since we know an individual receives determining characters twice over, one from each parent. The intermediate hybrid form in a crossing of these two forms will then be 'Vs' (where 'V' is the dominant character and 's' the recessive), which if crossed with the pure

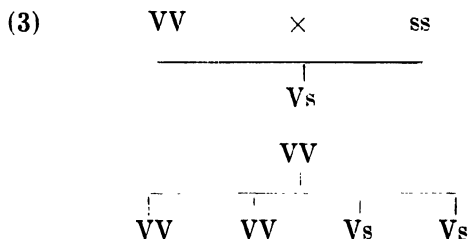
subpictus form 'ss', the possible combinations are 'Vs' and 'ss' with equal chances of each.



We would not have got 50 per cent *subpictus* forms, if it was a case of inbreeding amongst individuals of the first hybrid generation 'Vs', for then we would obtain 25 per cent *subpictus* only.



Neither was it a case of crossing between pure and hybrid *vagus* forms, for then the progeny would all bear *vagus* characters being 50 per cent pure and 50 per cent hybrid.



The discussion here is based on the supposition that *vagus* manifests the dominant characters. If, however, *subpictus* be considered as representing the dominant characters, the results would not be materially affected, for even then the assortment of the different forms will follow the same principle as enumerated above, with the change that the impure hybrid forms will all be *subpictus*-like. But which of the two types has in reality the dominant factor or factors in its characters, can only be settled after further elaborate experiments.

So far as I can find there is no previous record of similar occurrence in Anophelines. But Schuurmans Stekhoven and Schuurmans Stekhoven-Meijer

(1922) in Netherlands Indies noted some larvæ of the *vagus* type producing *A. subpictus* (*rossi*) mosquitoes. On the other hand, Treillard (1934) mentions of *A. subpictus* larvæ producing nearly 20 per cent *A. vagus* adults. There is therefore evidence that larvæ of either type may produce adults representing both the forms (i.e., *vagus* and *subpictus*).

These findings definitely indicate that interbreeding to some extent goes on amongst these two types of closely related mosquitoes, and possibly matings of the nature as represented in equations (2) and (3) above are more frequent; consequently this highly interesting phenomenon of hybridisation in these insects so long remained undetected. The question now arises as to whether or not we can cast doubt upon the validity of true specific ranks of the two types of mosquitoes *vagus* and *subpictus*, which, however, were at one time included under one composite species *A. rossi* (Swellengrebel and Swellengrebel, 1919; Christophers, 1933).

Further study is being conducted on this line, which when completed is expected to throw more light on the problem.

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SOME PECULIARITIES IN THE BREEDING HABITS OF THE COMMON *ÆDES* (*STEGOMYIA*) MOSQUITOES OF CALCUTTA.

BY

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[18th May, 1935.]

THERE are at least two species belonging to the genus *Ædes*, viz., *Ædes albopictus* Skuse and *Ædes aegypti* Linn. that are commonly met with in Calcutta. The prevalence of these two species in Calcutta has been long recognised by workers like Brunetti (1907) and Paiva (1912). Senior White (1934) also in a recent paper showed the relative abundance of the two species in the city.

Although adults of both these species are sometimes found together they have been known to differ considerably in their breeding habits. *A. aegypti*, for instance, prefers to breed in domestic waters in or around the houses, whereas *A. albopictus* is believed to be a sylvan species and breeds in tree-holes generally.

Certain deviations from these oft-noted breeding habits of the species have been observed by me only casually. In view of the importance attached to anti-stegomyia operations, for the simple reason that these mosquitoes are involved in the transmission of yellow fever and dengue, any peculiarity noted in the breeding habits of the species deserves to be recorded.

A. albopictus.

Barraud (1934), in his valuable work, puts down as the habitat of the species 'tree-holes, bamboos, leaf-axils, only rarely in artificial receptacles or rock-pools'. Senior White (1934) therefore considers the low prevalence of the species within his controlled zone as due to the fact that tree-holes in recent years have been filled with sand and asphalt.

However implicated the species may be with the arboreal mode of life, it is capable of breeding in artificial receptacles in Calcutta. My attention was first drawn to one such instance through the courtesy of Dr. H. N. Ray of

the Zoology Department, Calcutta University, who showed me certain mosquito larvæ breeding in a broken glass jar holding some rain water. These larvæ on examination turned out to be those of *Aedes albopictus*. Subsequently I detected the species breeding in a metal drum lying uncared for in the compound of a Calcutta building.

There is, therefore, evidence that the species in Calcutta does not restrict to tree-holes, but finds suitable breeding facilities in artificial receptacles as well.

A. aegypti.

A. aegypti on the other hand is essentially a domestic species breeding in household receptacles, and, although Barraud (1934) considers it unusual to find the species breed in tree-holes, ample evidence can be found to testify that a fair proportion of these troublesome mosquitoes in a place may originate from the tree-holes. Dunn (1927) for instance found that the species was breeding in nearly 27 per cent of the trees examined, and was occupying second place in frequency of occurrence. Again Hamlyn-Harris (1931) notes that 'In parks, public grounds, roadways, etc., trees situated within easy distance of dwellings show a tendency to breed *A. argenteus*,* rather than any other species'. In a survey of such trees we found 50 per cent positives of this species'. More recently Taylor (1934) has shown further evidence of the species breeding in tree-holes in Northern Nigeria. I have also at least on one occasion found the species breeding in a tree-hole in the environs of Calcutta.

But breeding of a more varied nature was revealed when larvæ of the species were recovered from the leaf-axils of *Dressina* plants in a Calcutta garden. Larvæ of this species were noticed growing in the little rain water that accumulated at the base of the partially opened leaves of these plants after a shower of rain.

Evidently the species is capable of adapting itself to any small accumulation of rain water, whether in tree-holes, leaf-axils or artificial receptacles.

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* *Aedes aegypti*.

OBSERVATIONS ON MALARIA-CARRYING MOSQUITOES OF CALCUTTA.

BY

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[31st May, 1935.]

INTRODUCTION.

THE following observations were carried out in the laboratory of the Mosquito Control Department of the Calcutta Corporation from 1st August, 1933 to 31st July, 1934. According to Dr. K. S. Roy's Mosquito Control Scheme, the Municipal area of Calcutta consisting of thirty-two wards (see Map I) has been divided into forty-two blocks, one mosquito sub-inspector being in charge of each. The mosquito sub-inspectors were asked to collect larvæ and adults of all types of mosquitoes from every part of Calcutta, and these were identified and recorded in the laboratory.

GENERAL SCHEME OF WORK.

Every morning the sub-inspectors with their block supervisors and workers, collected larvæ from all types of breeding places which they then either abolished or treated with kerosene or Paris green. The larvæ as well as a large number of adults were sent to the laboratory in separate tubes, and all necessary information regarding the nature of the breeding places and source of every sample was supplied to the laboratory.

PRINCIPAL FEATURES OF THE AREA.

(a) PHYSICAL.

Calcutta is situated 22° 34' N. and 88° 22' E. about 86 miles away from the sea on the bank of the Hooghly. The city covers an area of 31 square miles and is about 20 feet above the sea level. The soil is largely alluvial with layers of sand and clay. The level of subsoil water is high.

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(b) METEOROLOGICAL.

The annual average rainfall is 60 inches and the average number of rainy days per year is 118. Humidity averages 78 per cent of the saturation, ranging from 69 per cent in March to 89 per cent in August. The average temperature in the hot season is 85°F., in the rainy season 83°F. and in the cold season 72°F. The mean maximum temperature is 102°F. in May, and the mean minimum temperature is 48°F. in January. The meteorological data for the year under observation are given below :—

TABLE.

Month.	Amount of rainfall during the month (inches).	Average maximum temperature during the month (°F).	Average minimum temperature during the month (°F).	Average relative humidity taken at 4 p.m. during the month (per cent).	Number of rainy days.
1933.					
August ..	14.70	88.30	78.6	82	20
September ..	10.49	88.50	78.5	79	16
October ..	9.71	87.60	74.9	69	9
November ..	0.00	83.30	65.8	51	0
December ..	0.00	77.90	58.0	48	0
1934.					
January ..	0.00	77.50	55.7	40	0
February ..	0.08	83.60	61.9	35	0
March ..	0.16	94.70	70.7	27	1
April ..	1.95	96.10	77.6	50	4
May ..	2.55	97.0	79.3	57	4
June ..	7.69	91.8	79.2	74	9
July ..	8.91	90.2	79.3	81	18

(c) WATER SUPPLY.

The average daily supply of water during the year 1932-33 was 58,856,000 gallons of filtered and 50,902,000 gallons of unfiltered water. But filtered and unfiltered water are supplied at high pressure only at certain hours of the day, hence an enormous number of cisterns and receptacles for storing water are in use throughout the city. Drinking water is supplied to *bustees* (i.e., huts where filtered water connections are not usually allowed) by means of motor lorries in the morning. As stated in the *Corporation Year Book* for 1935 the number of unfiltered water connections was 44,783 and of filtered 54,616. Since the water supply is not continuously at high pressure, there is one or more storage cisterns for each connection. This shows the appalling number of man-made breeding places existing throughout the year in the city.

(d) DRAINAGE.

Underground drainage with water-carriage system of sewage disposal, exists only in the developed portions of the city, which comprise nearly a quarter of its total area. It may be noted that in 1924 some of the suburban municipalities were amalgamated with the Calcutta Corporation. These added areas,

consisting of wards 26, 28, 29, 30, 31 and 32, have surface drains only. There are altogether 320 miles of surface drains in Calcutta.

(c) COMMUNICATIONS.

Calcutta is the commercial capital of India. Railways, steamers, aeroplanes, country boats and motor buses are the principal means of connecting Calcutta with the different parts of India and the world. All these play an important rôle in the dissemination of mosquitoes. The districts surrounding Calcutta are malarious, and infected mosquitoes can easily reach Calcutta by any of these means of communication. Country boats, which carry goods and passengers to and from Calcutta, generally harbour a number of mosquitoes. *A. sundaius* was captured in some of these boats by the officers of the Bengal Public Health Department. The motor buses which ply between Calcutta and the neighbouring villages may likewise introduce dangerous mosquitoes into Calcutta. Three important railways converging in Calcutta are the East Indian, Bengal Nagpur, and Eastern Bengal Railways, and these pass through malarious countries before reaching Calcutta. There are two other light railways in Calcutta, viz., the Kalighat-Falta Railway and the Baraset-Basirhat Railway.

Thus a large number of mosquitoes, including the malaria-carrying ones, may easily be introduced into Calcutta from the neighbouring districts by means of railways, steamers, country boats, motor buses, etc. It may be noted that the establishment of two aerodromes, one at Dum-Dum very near Calcutta and the other in Calcutta itself, increases the possibility of introducing another dangerous mosquito-borne disease, viz., yellow fever.

PRESENT INVESTIGATION.

The following three species which are known to be malaria carriers in Calcutta were studied in detail :—

- (a) *Anopheles stephensi*.
- (b) *Anopheles sundaius*.
- (c) *Anopheles varuna*.

(a) *A. STEPHENSI*.

A. stephensi is the only mosquito found in Central Calcutta capable of transmitting malaria. Its larvæ are very common in the sewered areas of Calcutta. For some unaccountable reasons the adults of this species were difficult to catch. Out of 46,782 samples of mosquito larvæ caught during this investigation, 4,307 samples were *A. stephensi*.

BREEDING PLACES.

A. stephensi larvæ were found in many types of water collections, the following being the most favourite places :—

(i) *Cisterns*.—These are made of galvanised iron for storing water on roofs of houses. The majority of them contain unfiltered water for flushing privies. Every house with a sanitary privy must have at least one such cistern

on its roof, and there are many houses with four or five. Owing to low pressure and intermittent supply of filtered water, people collect water in masonry cisterns on the ground floor, and in some cases pump it into a cistern on the roof to get a continuous supply of filtered water throughout the day. This has again increased the number of storage cisterns on the roof. The total number of iron cisterns in Calcutta has been roughly estimated to be about one lakh. Many of these cisterns are without lids, some have their lids displaced, and some are considerably damaged. Consequently mosquitoes can easily enter into these cisterns to lay their eggs. The punch hole made by the Corporation to gauge the thickness of the galvanised sheet, as well as the warning pipe for the overflow of the surplus water, are also additional openings through which the mosquito gains entrance into the cistern. Cisterns, which had been newly installed or recently painted, were almost always found to be free from larvæ. In the course of this investigation 12,058 cisterns were examined, of these 2,893 were found breeding *A. stephensi*, i.e., 24 per cent were positive. The larvæ of this species were found in cisterns even on the roof of the fifth story.

(ii) *Masonry cisterns*.—So far as *A. stephensi* breeding is concerned, the masonry cisterns are next in order of importance. These are masonry structures for storing water on the ground floor. The water is stored for (1) domestic purposes or (2) trade and industrial purposes.

(1) Owing to intermittent nature of supply, water is stored in masonry cisterns. Every *pucca* house has at least one such reservoir and generally two or three for bathing, washing, cooking, etc.

(2) Water is stored in masonry cisterns for various trade purposes. Numerous masonry cisterns are found in jute mills, rice mills, oil mills, jute presses, tanning depôts, dyeing sheds, cattle sheds, motor garages, workshops, etc.

Those masonry cisterns, whose water is not changed frequently, breed mosquitoes, and in this respect the cisterns used for industrial purposes are a great source of mosquito breeding. Similarly, when a house has two or more masonry cisterns one is used and is regularly cleaned, while others not being cleaned become a source of mosquito breeding. Out of 4,656 masonry cisterns examined, 745 were found breeding *A. stephensi*, i.e., 16 per cent were positive.

(iii) *Temporary collections of water* are next in order of importance so far as *A. stephensi* breeding is concerned. Rain water collects in broken tins, mugs, earthenware vessels, coconut shells, glass phials, cups, empty pitch barrels, etc., lying on the roof or in the yard of a house. During the rainy season the number of temporary collections of water increases very much and the increase of *A. stephensi* in this season is partly due to this.

(iv) *Other breeding places*.—So far as *A. stephensi* production is concerned the other breeding places are not very important. *A. stephensi* larvæ have been found in fire buckets, garden fountains, and in one instance in a drain containing fairly clear water. *A. stephensi* larvæ have also been found in a few instances in tanks.

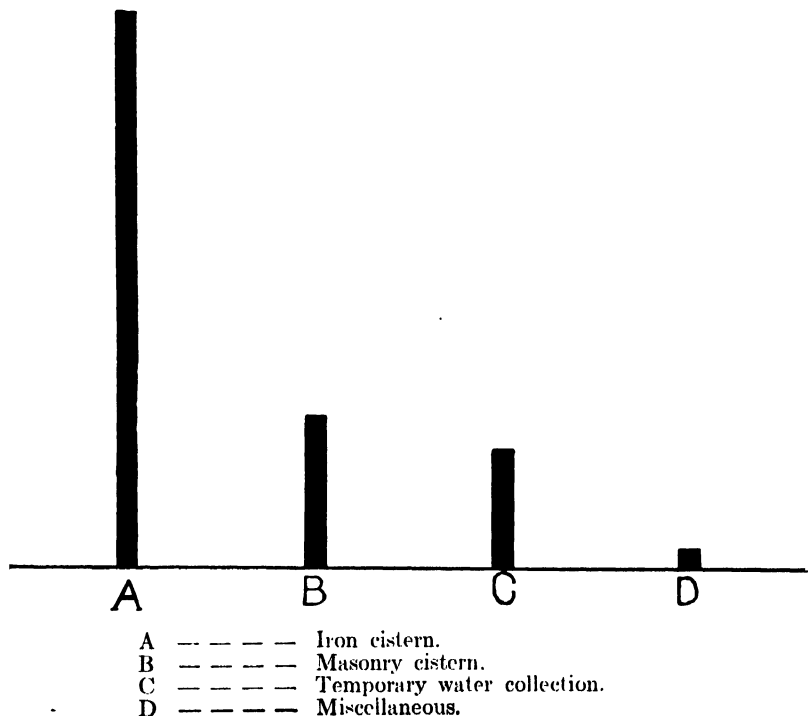
RELATIVE IMPORTANCE OF THE BREEDING PLACES.

During this investigation 4,307 samples of *A. stephensi* larvæ were received in the laboratory; each sample containing 25 larvæ on an average; out of this

2,894 samples, *i.e.*, 67·2 per cent, were collected from iron cisterns, 745 samples, *i.e.*, 17·3 per cent, from masonry cisterns, 581 samples, *i.e.*, 13·5 per cent, from temporary collections of water and 86 samples, *i.e.*, 2 per cent, from all other breeding places including fire buckets, garden fountains, etc. Chart I shows graphically the relative importance of each type of breeding place.

CHART I.

A. stephensi breeding in different types of water collections.



SEASONAL PREVALENCE.

There was a marked increase in the number of *A. stephensi* in the rainy season, and the maximum was reached in August. Six hundred and eleven samples, *i.e.*, 14·2 per cent of the catch for the total period, were received in the laboratory during the month of August. After the rainy season the number gradually declined and the minimum was reached in December during which 150 samples, *i.e.*, 3·5 per cent of the total catch of *A. stephensi* larvæ, were received in the laboratory.

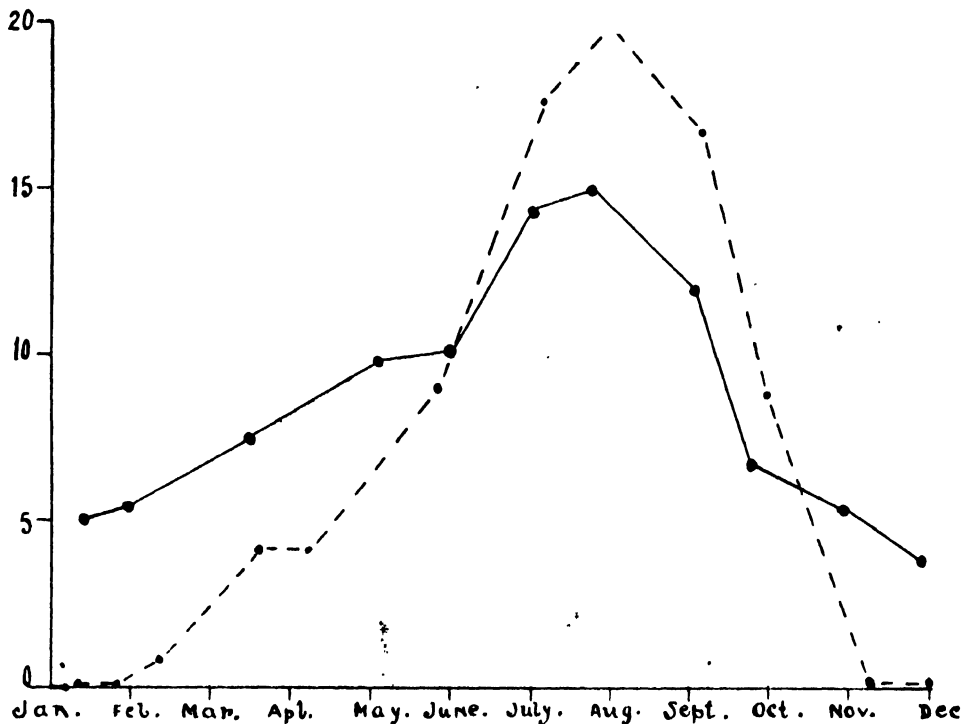
Chart II shows the prevalence of *A. stephensi* breeding month by month, and illustrates the relation between the number of *A. stephensi* larvæ caught in each month with the number of rainy days in that month. Here, as will be seen from the graph, the correlation is definite. It is evident that the number

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of *A. stephensi* in each month does not depend so much on the amount of rainfall as on the number of rainy days in that month.

CHART II.

Correlation between *A. stephensi* breeding and the number of rainy days per month.



--- represents number of rainy days in the month.

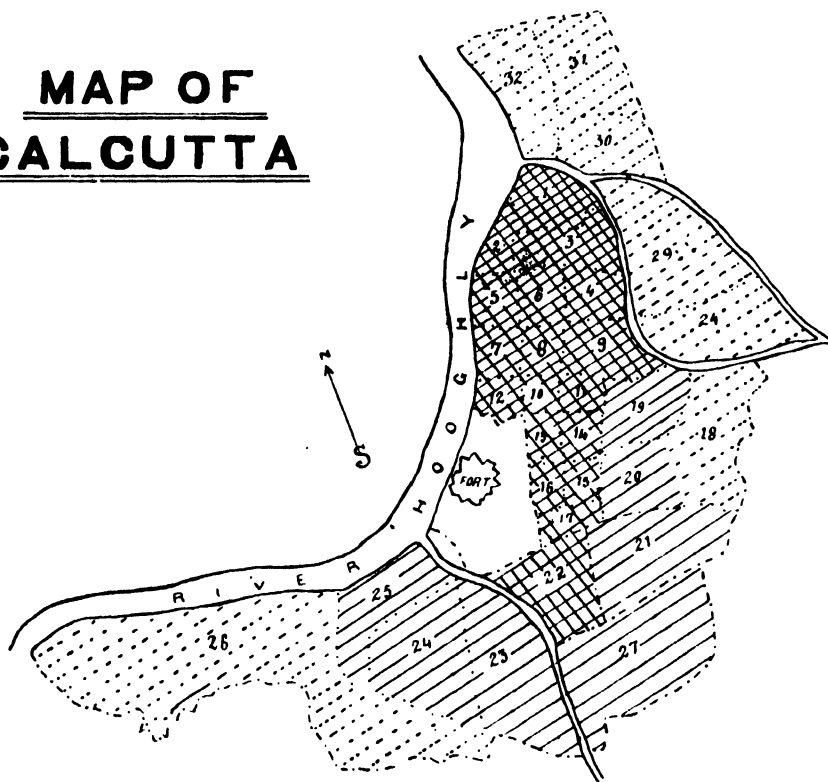
●—● represents the percentage of *A. stephensi* caught, e.g., in August the number caught is equal to 14.5 per cent of the total year's catch.

DISTRIBUTION OF *A. stephensi* IN DIFFERENT PARTS OF THE CITY.

In this connection it is advantageous to remember that only certain parts of Calcutta, comprising about one-fourth of the total area of the city, enjoy a water-carriage system of sewage disposal and are provided with an unfiltered water supply. *A. stephensi* is very prevalent in the sewered area, and the larvæ were found mainly in the unfiltered water cisterns. There are other less developed portions of the city enjoying filtered water supply alone, without underground sewers and unfiltered water supply; here the prevalence of *A. stephensi* is much less. The most backward areas of the city, e.g., portions of wards 26 and 31, have neither filtered nor unfiltered water supply and these are practically free from *A. stephensi*. This species is very rare in the greater

MAP I.

MAP OF CALCUTTA



Showing intensity of *A. stephensi* breeding ward by ward.

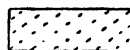
Intense amount of *stephensi* breeding



Moderate amount



Very small amount



parts of wards 18, 27—30 and 32 (*see* Map I). There are certain other wards, *e.g.*, 19, 20, 21, 23, 24 and 25, which are partly sewered, and *A. stephensi* is confined to the sewered portions only.

(b) *A. SUNDAICUS.*

This species was found only in the eastern border of the city, near Salt Lakes in wards 28 and 29. Its larvæ have been found mainly in tanks. Though *A. sundaicus* is reported to be a salt-water breeder, yet in almost all instances it was found breeding in water which, strictly speaking, cannot be called saline. Most of the tanks contained floating algæ, *e.g.*, *Oscillatoria germinata*, etc. Altogether 207 samples of *A. sundaicus* larvæ were caught. These were often found in tanks used for rearing fish spawn. In a few instances they were caught from the Belliaghata and the Shambazar Canals. They were most prevalent during and immediately after the rainy season. This species was responsible for an outbreak of malaria in ward 29 in July 1933.

(c) *A. VARUNA.*

It was found mainly in the southern and south-eastern parts of Calcutta. Altogether 184 samples of its larvæ were captured in the course of this investigation. Almost all the larvæ were caught in wards 21, 26 and 27. Large tanks with marginal vegetation were the chief breeding places. No larvæ could be found in tanks with clean edges, *e.g.*, in Dhakuria lakes in ward 27 which is kept clean by periodic removal of the marginal aquatic vegetation. During the rainy season these larvæ were also found in the slowly moving water of the roadside drains. The adults were found mainly in huts occupied by human beings. The majority of the larvæ were caught during and immediately after the rainy season.

SUMMARY AND CONCLUSIONS.

1. *A. stephensi*, *A. sundaicus* and *A. varuna* are the only species transmitting malaria in Calcutta.

2. *A. stephensi* is much more prevalent than the other two. The distribution of this species corresponds with the sewered area of the city.

Storage cisterns are responsible for 84·5 per cent of *A. stephensi* breeding. This species is most prevalent during the rainy season and least common in winter.

The amount of *A. stephensi* breeding depends not on the amount of rainfall but on the number of rainy days in a month.

3. *A. sundaicus* is at present confined to the eastern border of the city.

It breeds mainly in tanks, and was responsible for an outbreak of malaria in ward 29 in July 1933.

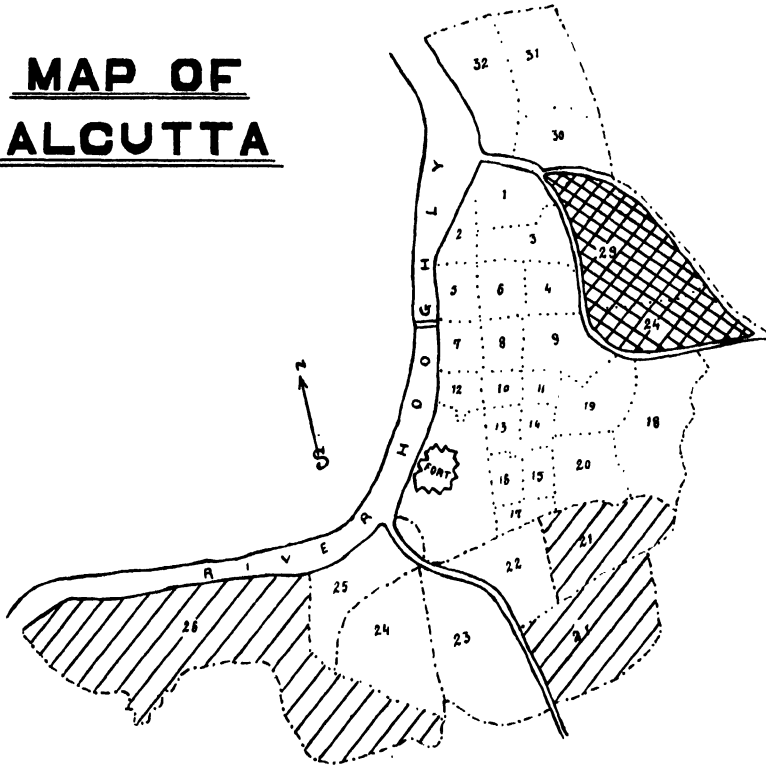
4. *A. varuna* is confined to southern and south-eastern parts of the city. Large tanks with marginal vegetations were the chief breeding places.

* * * * *

My thanks are due to Dr. K. L. Chowdhury, Mosquito Controlling Officer, Calcutta Corporation, for his valuable suggestions and encouragement and to Dr. A. T. Bose for his valuable help.

MAP II.

MAP OF CALCUTTA



Showing distribution of *A. sundaeicus* and *A. varuna*

A. sundaeicus is found in large number — — — — —



A. varuna — — — — —



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WHAT MALARIA COSTS INDIA, NATIONALLY, SOCIALLY AND ECONOMICALLY.

BY

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FOREWORD.

THE subject of the losses due to malaria, by reason of both its direct and indirect actions, is so vast that it is impossible in an article of this nature to do more than touch briefly upon the very varied aspects of this many-sided problem. Some of the more relevant observations from the literature available have been collected in relation to the malaria problem in India. There are undoubtedly many others, equally important, scattered through the records of the Public Health Departments of the different provinces of this country, that would shed more light on this subject. It is hoped that the data and discussions given here may stimulate a more detailed and precise study of the question, in its relationship to the malaria problems of different localities and provinces in India.

The necessity for drawing attention to the grave danger which malaria places in the way of the progress of India, was one of the reasons for the compilation of this article. It is hoped that in future a much greater interest will be taken, by both the lay and the official populations, in a disease which is sapping the physical vitality of the people, the social and intellectual development of the nation, and the economic resources of this sub-continent. It is hoped that a realisation of the importance of malaria to India will lead to a better and more widespread knowledge of the disease, and the initiation of more forceful and active measures for its amelioration. It is also hoped that the information given here may prove of value to those workers who have to represent to the financial authorities the necessity, the urgency, and the economic importance of anti-malarial measures*.

The plea of the sanitarian and of the malariologist is too often as 'the voice of one crying in the wilderness'. India is like Prometheus bound by chains of apathy to the rock of expediency and financial stringency, while the vulture of malaria devours the vitals of her people.

A. INTRODUCTION.

'Malaria is one of the greatest scourges inflicted upon humanity. It's a menace to any people or country in which it has a decided incidence. In the number of deaths caused either directly or indirectly, the sickness and suffering, the loss of time and efficiency, the expense, the lowered vitality of the afflicted, and the reduction of the valuation of property, malaria is without rival among the diseases afflicting mankind' (Leathers, 1918).

'No disease that ever afflicted mankind can exceed in its toll of death, suffering, invalidism, wrecked nations and civilizations, the ravages of malaria. This disease is still the major enemy of the tropics, and entitled to be called "Captain of the Men of Death" Greece and Rome fell beneath its sway. Its cost in money and lives is stupendous' (Reed, 1929).

'It has been said that one-half the mortality of the human race is due to malaria. This may very well be an exaggeration, but there can be little doubt that of all the ills that flesh is heir to, malaria is the most deadly, and exercises the most profound influence on the distribution and activities of man' (Shipley, 1908).

'Malaria stands first on the list for injury it does to the community' (Carter, 1919).

'It (malaria) is the prevalent fever of Ceylon and causes more deaths, ill-health, degeneracy and misery than any other disease with which the inhabitants are afflicted' (Nicholls, 1924).

*In order that local data may be available for reference by such workers, in many places information has been given in greater detail than appeared absolutely necessary for the discussion of the subject.

The statements quoted above may appear very extreme in connection with a disease like malaria, which is so universally present in India, and which, at ordinary times, excites comparatively little comment among the lay public. Very similar views have, however, been expressed by authoritative observers both in the Indian Legislature and in the medical press of this country.

Some of the opinions which have been placed before the Government of India on the serious nature of this problem, are given below.

A resolution of the Department of Education, dated 23rd May, 1914, reads as follows :—

‘The most important tropical disease in India is malaria. After allowance has been made for the tendency to attribute to fever deaths from other causes, malaria stands out as universally prevalent in India and in many tracts it is a scourge greater than either plague or cholera. It maims as well as kills, and causes more sickness, misery and death than any other single disease’.

The Hon’ble Mr. Surendra Nath Banerjee placed a resolution on malaria before the Imperial Council in 1916. In presenting this resolution he said ‘we have the plain fact admitted by the Government of India (*vide* above resolution) that malaria is the greatest scourge that decimates the people of India.....The loss of human life does not alone represent the sum total of the suffering and misery inflicted on the people by malaria. For one man who dies of malaria at least ten suffer from it—though they do not die of it. They carry on a lingering existence hovering between life and death, to whom death in many cases would be a relief.....Having regard to the terrible mortality caused from malaria and the wide area it covers, I think I am entitled to hold that it has become a grave national problem’.

The Hon’ble Khan Bahadur Dr. Sir Nasarvanji Choksy in proposing a resolution on the treatment of malaria at a meeting of the Council of State in 1933, remarked :—‘The resolution I have the honour to move embraces a triple tragedy: firstly the tragedy of the masses who suffer and succumb to the ravages of malaria; secondly the tragedy of the large stocks of quinine that Government cannot dispose of; and thirdly, the tragedy of the quinine policy of the Government.....As regards the masses, malaria is almost universally present throughout the greater part of India.....Thus one out of every 3·5 deaths in India may fairly be assigned to this cause alone.....Malaria, it has to be noted, is an affection that does not kill at the first attack, except in the most malignant type. It is, however, the recurrent attacks in untreated and partially treated cases that sap the vitality of the masses, undermine their constitution, reduce their physical stamina and create in them a state of chronic debility that prevents the full economic value of the peasantry and industrial workers. Productivity of the soil thus becomes greatly reduced, and economic distress, poverty, disease and death follow in its wake. Apart from that, they fall an easy prey to other diseases and die. As the cause of death is registered for the final illness alone, the underlying cause—malaria—becomes entirely masked. They also produce a debilitated progeny that increases the infantile mortality’.

Christophers (1926), whose knowledge of malaria in India is unsurpassed, says ‘whether from the point of view of enhanced mortality, sickness and individual suffering, or from the effect of preventing natural increase and sapping the vitality of populations, or the paralysing effect on industry and

exploitation of the mineral or other natural wealth of the country, or in the direct loss to Government in a variety of ways, malaria is universally recognised as the most important sanitary problem with which India has to cope'.

The Public Health Commissioner with the Government of India in his Annual Report for 1930 considers this disease to be 'one of the most disastrous causes of human distress and economic loss in India'. While Hehir (1927) states that 'it is malaria which is the main cause of the insalubrity of India, and so long as it continues prevalent, widespread and severe, so long will it sap the vitality of the people'. The All-India Research Workers Conference held at Calcutta in 1924, in deciding upon a priority programme of research into the diseases of India, placed malaria first on the list.

In spite of these awesome and authoritative opinions, but a few of those expressed about the serious nature of the malaria problem, the disease is usually looked upon with a considerable degree of indifference by the population of this country. 'The inhabitants accustomed to the disease, and perhaps influenced by the fatalism which so often accompanies it, have not proclaimed from the house-tops their unhappy condition' (Jones, 1909). Few persons, either among the educated or the uneducated classes, appear to realise the enormous ravages which malaria causes, both directly and indirectly. We see it about us every day and *comparatively* little notice is taken by the lay public, the press, or the authorities, who become seriously alarmed if a case of cholera occur, or if plague be present. Mackie (1925) estimates that, during the 20 years when the epidemic of that dreaded disease, bubonic plague, was at its height in this country, it killed 10 millions of people, *i.e.*, an average of half a million a year. Yet malaria, even in the absence of epidemics, is probably responsible directly for over a million deaths each year, and this awful state of affairs has continued, and probably will continue, for many generations, unless some drastic steps be taken in the matter. It must also be remembered that this great mortality occurs, apart from an indirect death rate which is probably as great again.

At the present time, the Public Health Commissioner with the Government of India states that, in 1931, the combined effects of cholera, plague and small-pox were responsible for nearly 5 out of every 100 deaths. As compared with this, 60 per cent of the total mortality is ascribed to 'fevers', of which about one-third to one-fourth are probably deaths *directly* due to malaria (*vide infra*). This means that, at the present time, malaria is responsible in an ordinary year for the direct demise of about 3 or 4 times as many people as the other three major epidemic diseases combined. When, however, one of those fulminant epidemics of malaria occurs, even this high death rate is very greatly enhanced, as was the case in 1908.

Apart from their mortality, the other major epidemic diseases kill rapidly. They do not usually leave behind them that trail of chronic disease and debility which is such a special feature of malarial infection. It is probable that these sequelæ are ultimately responsible for even more damage than the direct mortality. Indeed, as stated by Balfour and Scott (1924), 'it is what we may call the everyday malaria which is the truly "Imperial" disease, the chronic relapsing malaria which saps life and energy, alters mentality and leads to invalidism and poverty'.

The reason for the indifference of the public is apparently because in the case of plague, cholera and smallpox, the dramatic nature of the deaths is very

impressive. On the other hand, except in those rare fulminant epidemics, such an element is usually absent from a malarial death, and the havoc caused by the disease is of a much more insidious character. Yet, as mentioned above, the ravages of these other epidemic diseases are small as compared with the devastation wrought by malaria in India. The occurrence of plague, cholera or smallpox in any country becomes at once an international health problem, because of the infective nature of these diseases. The Government of the country concerned is forced to take serious notice of their occurrence, because of the international repercussions caused by any marked incidence. Malaria on the other hand may be considered largely as a purely domestic disease of the country concerned.

The toll which malaria takes of the population, the wealth and the resources of India, may be considered under the following headings :—

- (1) The effect upon the natural increase of the population.
- (2) The effect upon the health, vitality, and physical development of the people.
- (3) The effect upon the social, intellectual and political progress of the nation.
- (4) The effect upon the economic, agricultural, and industrial development of the country.

When these factors have been considered, it is then necessary to determine whether any estimate can be made as to

- (5) the financial loss, direct and indirect, which this disease causes to India, and
- (6) whether it is a paying proposition to undertake measures for the control or amelioration of the disease.

B. THE EFFECTS OF MALARIA UPON THE NATURAL INCREASE OF THE POPULATION.

‘Wherever there is a rise in death rate it is accompanied by a rise in birth rate—that is the beneficent ordering of nature. If it were otherwise, depopulation would follow. But in Bengal this wholesome law of nature is reversed, or, at any rate, suspended’ (Resolution re Malaria. Banerjee, 1916).

‘There is very marked correlation between the rates of growth of the population of Bengal and the prevalence of malaria’ (Bentley, 1916).

‘In hyperendemic and severely endemic districts of India, the tendency is, on the whole, for the population to decline, whereas in healthy districts, *cæteris paribus*, there is a moderately rapid rise in population’ (Hehir, 1927).

‘Malaria is therefore the major cause of the depopulation of areas infected by it: this is not surprising inasmuch as the disease not only weakens the human system and often causes the death of men.....’ (Celli, 1933).

Various workers have commented upon the action of malaria in retarding or stopping the natural rate of increase among populations in parts of India where the disease is prevalent.

Periodically severe regional epidemics of malaria occur in different parts of India, more especially in the north-western portions of the country*. These

*The ‘Malaria Map of India’ prepared by Christophers and Sinton (1926), gives a general idea of the distribution and intensity of malaria in the various parts of the country.

epidemics may have such great effects in increasing the mortality and morbidity of the population afflicted, as to cause serious alarm both to the lay public and to Government.

The most striking examples of these epidemics occur in the Punjab, where they have been specially studied by Christophers and by Gill. Newell (1913) also pointed out that the effects of malaria in the Punjab, directly and indirectly, sap the growth of the population.

Christophers (1924) records that, during the great epidemic of 1908 in this province, a quarter of a million people out of a population of 20 million died inside the three months when the epidemic was at its height. Gill (1928), when referring to the effects of this epidemic on the people of Amritsar, calculates that, apart from premature deaths, miscarriages and abortions during the last 4 months of the year, malaria caused the natural increase of the population to be checked by 792 deaths in this city alone. This is equivalent to about 5 per mille of the population.

In this connection Christophers (1911b) states that 'it is chiefly to epidemics that we must look for the effect malaria has had in causing a decrease of the population in certain areas (of the Punjab), or preventing the normal increase in others. It is not only rural areas that suffer, for the smaller towns have often suffered terribly, whenever involved in an epidemic area; even the larger cities do not escape, Amritsar having been on two occasions nearly decimated by epidemic malaria'. The same author (Christophers, 1911a) says 'the condition of a population in the Punjab as regards natural increase or decrease is influenced rather by the number and severity of the epidemics to which it has been exposed than by its endemic malaria'. Gill (1917) reports that 'malaria is the most important disease exercising a check on the natural growth of the population at Amritsar'. In addition to its effects on the total death rate and on the infantile mortality, malaria also checks the natural increase of the population by lowering the birth rate. This is especially marked in the year following one with high 'fever' death rates.

Epidemics, such as those studied by Christophers and by Gill, may cause the natural increase of the population to stand still for several years, and may even be felt at a later date when the younger generation, wiped out by the epidemic, would have reached child-producing age.

The harmful effects of epidemic malaria are most spectacular, and usually give rise to great popular outcry. The action upon the natural increase of the population is, however, often only a temporary check, and the normal rate of increase of the survivors may be quickly re-established (Gill, 1928). On the other hand, endemic malaria is less conspicuous in its action. It is so widespread in India that the lay public have become indifferent or resigned to the damage it causes, considering it to be part of the normal course of events. It is, however, a wolf which is always gnawing at the vitals of the people, and, in the long run, almost certainly causes very much more damage to the health and lives of the people of India than does epidemic malaria. The damage caused by this insidious action must therefore be considered.

Banerjee (1916) says 'it would be no exaggeration to say that some of the fairest parts of my loved province (Bengal) have been decimated by this terrible scourge'.

Bentley (1925) has made a special study of the malarial conditions of Bengal, and has recorded many very interesting observations on the growth of the population in different parts of that province. Of the natural divisions of Bengal, the Western and Central ones are the most malarious, while the Northern and Eastern are less so. This author reports that in Eastern Bengal,

where malaria is on the whole comparatively slight, both the birth rate is higher and the death rate lower than in any of the other three divisions. 'As a direct result, the rate of natural increase of the population in Eastern Bengal was very much higher than elsewhere, and nearly eight times greater than that of the Burdwan Division (Western Bengal)'. 'The total expansion of population in Eastern Bengal during the decade (1901-1911) was over 12 per cent, a figure far in excess of that in any of the other three natural divisions'. 'For many years past, the growth of population in Western and Central Bengal has undergone a serious check owing to diminished birth rates and excessive mortality. The factors mainly responsible for this condition are malaria and economic stress resulting from agricultural decline'. 'These factors are known to be specially destructive of child life, and the proportion of children under ten in the different natural divisions reflects their influence very clearly in the case of both Western and Central Bengal'. Table I has been compiled from the work of Bentley (1925).

TABLE I.
Statistics relating to malarial prevalence in Bengal.

Division.	Fever index* (per cent) (1911).	Estimated total malarial infections (millions)†.	Estimated severe malarial infections (millions)†.	Mean recorded birth rate per 1,000 (1901-1910).	Percentage of children in population.	Percentage of children under 10 years in population in 1911.	Increase of children under 10 since 1901 (per cent).	Children under 10 to married women, aged 15-40.	Mean annual death rates per 1,000 corrected for migration.	Percentage increase in population (1872-1921).	Mean annual rate of increase per 1,000 corrected for migration.	Increase in density of population per square mile (1872-1921).
Western	40.9	8.0	5.25	33.0	25.9-27.2	25.5	2.1	1.5	31.8	5.7	1.2	25
Central	32.3	8.5	3.55	33.8	27.1-30.3	28.0	4.5	1.7	30.5	27.1	3.2	115
Northern	23.7	8.5	1.05	38.8	26.6-35.0	31.3	11.4	1.9	32.0	28.8	6.7	120
Eastern	7.5	5.0	0.50	37.5	29.1-34.7	32.0	13.1	1.9	28.3	69.8	9.2	336

* The 'fever index' here means the proportion of cases of malaria to the total number of cases treated in hospitals, etc.

† The population of Bengal at the 1911 census was 45,483,077.

The number and composition of the population of the different natural divisions of Bengal in relation to the incidence of malaria have been summarised in Table I. The extraordinary increase in the Eastern division, where malaria is comparatively mild, is due entirely to natural growth by excess of births over deaths. While the other divisions have gained in population to a greater or less extent by immigration, Eastern Bengal has lost rather than gained by

this process, because it has few large towns and few important industries to attract people from other areas.

Kenrick (1911) reports that the total population of the Central Provinces had increased considerably in the decade 1901—1911, but mainly in the non-malarious areas. 'On the whole hyperendemic villages show very slight increase, while very many show a decrease'. In a later paper (Kenrick, 1914), he instances certain villages which have become depopulated since the previous census.

Robertson (1909) pointed out the very deleterious effects of hyperendemic malaria upon the natural increase of the immigrant population of the Terai of the United Provinces. Phillips (1925) found that, in the Gadarpur Tehsil of this area, the estimated population in 1881 was 18,982, and since that time there had been a steady decrease. The population had fallen to 14,723 in 1901, to 12,526 in 1921 and was only 8,962 at the time he was writing. Clyde (1931), in speaking of the Kashipur district of the same area, remarks that if it were not for fresh immigration depopulation would occur automatically. The birth rate is only 35.7 per mille as compared with a death rate of 45.3 per mille. He concludes that 'any attempts at reclamation under the present (malarious) conditions, however, can never hope to be successful, as they are dependent on a constant immigration to keep up the population'.

The malarial conditions of Bombay City were investigated by Bentley (1911). He found that the Parsi community showed more evidence of chronic malaria than any other in the city. Among these people the birth rate had been exceedingly low for many years, and in a number of years deaths had been in excess of births.

In the malarious tea gardens of the Bengal Duars, Bentley (1908) states that there was a frightful loss of life by deaths among children and by miscarriages. This keeps the population (mostly immigrant) from increasing naturally. 'But for this the tea districts should have been self-supporting, as far as labour requirements, years ago'.

The labour in the tea gardens of Assam, as in the case of the Bengal Duars, is mainly supplied by the recruitment of immigrants. This has been going on for many decades, and it has been the aim of each garden to foster the natural increase of such population. So eventually it was hoped to do away with the necessity for this expensive method of recruitment from outside areas, and make the gardens self-supporting in this respect. This objective has not yet been attained in the majority of gardens, and the main obstacle to this achievement has been the baneful effects of malaria. 'Malaria is now recognised as the most serious cause of mortality and debility in Assam' (Ann. Rept. Public Health Commissioner, Govt. of India, for 1931).

Apart from the effects of malaria in influencing the natural increase of the population directly through its action upon the birth and death rates, it has another important indirect one.

As pointed out by Celli (1904) 'a certain correlation exists between emigration and malaria, in the sense that the regions where this pestilence is most prevalent are those which contribute largely to permanent emigration'. This action has also been emphasised by Watson (1924), who says 'quite apart from the sickness among men, the women are concerned with the sickness and loss of their children, and where the greatest primal instinct, the maternal instinct,

is thwarted there can be no rest or stability among the people. Loss of, and sickness among, children unsettle women. They instinctively flee from the place. And what the woman thinks to-day, the man thinks to-morrow'.

Kenrick (1914) also speaks of hyperendemic areas in the Central Provinces which are becoming depopulated as the aborigines emigrate to more congenial surroundings.

It is clear from the evidence and opinions reported above, that both epidemic and endemic malaria exercise directly a marked check upon the natural rate of increase of the population in those areas where it prevails. This direct action may be due to the effects of malaria upon (I) the birth rate and (II) the death rate. The mode by which these factors influence the natural increase of the population must be considered in greater detail.

(I) THE EFFECTS OF MALARIA UPON THE BIRTH RATE.

The detrimental action of this disease upon the birth rate was commented upon by Hippocrates. The birth rate of a population is influenced adversely by the prevalence of either epidemic or endemic malaria, but the effect in the former instance is usually only a temporary phenomenon from which recovery is rapid.

Kenrick (1911, 1914) in the Central Provinces, Horne (1913*a*, 1913*b*) in the Madras Presidency, and Gill (1928) in the Punjab, have called attention to the marked reduction which occurs in the average birth rate of the year following an epidemic. This reduction begins about 9 months after the commencement of the fever season, and is at its maximum about 9 months after the epidemic was at its height. There is, however, no appreciable decline in the year of the epidemic.

This subject has been specially studied by Gill (1928) in relation to the malarial epidemics of the Punjab. This author points out that the absence of the effect on the birth rate during the year of the epidemic is due to the fact that abortions, etc., during the months of severe disease, have little effect upon the total birth rate for the year. Epidemic malaria is at its height during the later months of the year, at about the time when the birth curve is at its maximum in normal years. The births occur, very often prematurely, and, being included in the vital statistics, make the number of recorded births up to the normal average in the epidemic year. The diminished birth rate in the following year is explicable by the number of early interruptions of pregnancy, and by the temporary cessation of conception due to the universal illness at the time of the epidemic. Gill (1928) shows that, during the second year after the epidemic, the annual birth rate returns to normal, or may even exceed this. This is due to the comparatively low fatality rate among adults of child-producing ages, and the abnormal number of women of this age, who are in a condition to conceive, because of abortion, miscarriage and sickness during and after the epidemic.

Although the effects of epidemic malaria may have a disastrous result for the moment upon the birth rate of the afflicted population, these effects quickly pass off and in a couple of years the rate among the *surviving* population returns to normal. On the other hand, the effects of endemic malaria, especially if the condition be hyperendemic, are much more disastrous, because its action is not a mere transient phenomenon but is practically continuous.

The remarks of Banerjee (1916) and of Bentley (1925) upon the effects of this type of malaria upon the birth rate in Bengal have been quoted earlier in this paper. Watson (1924) also points out that when a non-immune people get into an intensely malarious area, the women rarely become pregnant, and if they do they are very likely to abort. The birth rate under such circumstances may be so low as almost to disappear. Watson (1933) also mentions the instance of a coffee estate in Malaya which had to be abandoned after 5 or 6 years because of the severe malaria. During that time, although there were fifty women on this estate, not a single child was born, the malaria parasite having killed them all before birth. Barrowman (1934) notes that as the result of 30 years of anti-malarial work in certain places in the Federated Malay States, the birth rate has risen from 5 to more than 200 per 1,000 adult females.

Bentley (1911) in his investigation of Malaria in Bombay City states that 'it is well known that malaria exerts considerable influence in reducing the births and increasing the number of still-births that occur in a community'. 'It may be remarked also that, as a whole, the births in portions of the City known to be malarious have either remained stationary or show a slight decrease in recent years. During 1908 the ratio of still to live births increased'. 'It may be pointed out that the birth rate among Parsis, who as a race show more evidence of chronic malaria than any other community in Bombay, has been exceedingly low for many years. The Parsi birth rate has only once reached 10 per 1,000*; for long periods their total births have only exceeded the deaths by a small number, the maximum excess of births over deaths has only once approached 6 per 1,000; and for a number of years deaths have exceeded births'.

It was found by Robertson (1909) that a lower birth rate was recorded among the immigrant population of the malarious parts of the Terai than among the indigenous people†. This he attributes to the greater susceptibility to malarial manifestations of the former type of people. Similar observations are reported by Phillips (1925) and Clyde (1931).

Kenrick (1914) noted that in the Central Provinces the birth rate in hyperendemic areas may be as high as, or higher than, that in healthy areas. The inhabitants of the former areas are, however, mainly aboriginal or jungle tribes who are well known to be very prolific, and who also appear to be less seriously affected by malaria†. The birth cycles in these two types of locality were found to be markedly different. The annual birth curves in healthy areas reach their maxima in October or November, i.e., about 9 months after the spring, when the harvest has been gathered and the physical and material condition of the people is at its best. In hyperendemic areas, on the other hand, the annual curve of births reaches its maximum from March to May, because the most malarious months are from December to May, and fertile intercourse is in

* The annual birth rate per mille for British India is between 34 and 35. .

† The effects of malaria upon a population seem in many instances to be influenced considerably by the racial origin of the people affected. The indigenous or aboriginal tribes, who survive in many of the hyperendemic malarious tracts of India, appear to be much less seriously affected by malarial infection than the populations of Aryan and Dravidian origin who attempt to colonise such tracts. There seems to be much evidence to suggest that the former people have not only some degree of natural immunity to the manifestations of this disease, but also possess a more rapid, and possibly a greater, power of developing immunity to infection with it.

abeyance for some months until the season is more healthy. Kenrick states that the lower the degree of malarial endemicity of any place the more closely do the birth curves approximate to those of healthy areas.

From their investigation of malarial conditions in some areas of the deltaic portion of Bengal, Stewart and Proctor (1907) conclude that 'the diminished birth rate in these districts is also to be attributed at any rate in part to the great prevalence of malaria, acting both by causing abortion or still-birth, and by lessening the reproductive powers in persons debilitated by continuous attacks'.

The influence of malaria upon the birth rate may be due to two factors: (a) a diminution in the number of conceptions, and (b) the effects of malaria upon the duration and completion of pregnancy.

(a) THE EFFECTS OF MALARIA IN PREVENTING CONCEPTION.

Any diminution, either temporary or continuous, of the conception rate may be due to two causes, (i) a diminution of the sexual impulse amongst the individuals of a population weakened by the disease, or (ii) cessation or diminution of fecundity or virility of the parents, due to the same cause.

As mentioned above, the marked effects of acute malarial infections upon the number of conceptions are strikingly demonstrated by the effects of epidemics of malaria upon the subsequent birth rate. That a temporary cessation of conception results, is proved by the marked diminution in births which occurs nine months after the height of the epidemic. That this result is but a temporary event is shown by the fact that during the second year after the epidemic the birth rate returns to normal or exceeds it. A similar diminution in the number of conceptions has also been noted during the more malarious months in hyperendemic areas.

White (1909) considers that a diminished virility of the men may follow epidemic malaria in the United Provinces. With regard to the effects of chronic malaria in this respect, very many years ago Dyson (1895) pointed out that 'one of the gravest evils, and one which is particularly felt by Hindus, is the impotency so commonly found in water-logged villages, which results from the deterioration of the health produced by constant attacks of fever and the presence of an enlarged spleen. The men in these villages freely acknowledge it, and beg for some medical remedy'. Robertson (1909) stated that the early onset of impotence (commonly at about 30 years of age) is a byword in the malarious parts of the Terai of the United Provinces. 'Early impotence is well known to be constantly associated with malaria, and the assumption that the two are in direct correlation in the Terai seems warrantable, especially as we have made careful enquiries on this point and can discover no other adequate reason'. Gill (1928) also notes that in the water-logged areas of the Punjab, where malaria is rife, the men make the same complaint. This condition was reported in similar areas along the Western Jumna Canal by Taylor (1870)—'The miserable disease engendered by the tainted water and malarious exhalations of the soil, affection of the spleen, is very prevalent, and whilst it produces in its victims a listlessness and lassitude in the ordinary occupations of life, it deprives them in many cases of the hope of a family, which as is well known is on religious grounds one of the most trying afflictions which the Hindoo has to bear'. 'The unfruitfulness of women is a subject of common remark, and

the consequent difficulty of inducing other Jat families to give their daughters to the men of Panipat, and the environs of the canals generally, is very great.....'.

There seems little doubt that severe or chronic malaria may have a damaging effect upon the virility of the male.

The effect of malaria upon the fecundity of the female has long been recognised. Weatherley (1895) reported at the Medical Congress at Calcutta in 1894, that sterility is very common among the women of India. He lays this at the door of malaria. Laffont (1912) also attributes diminished fecundity among the women of Algeria to this cause.

Robertson (1909) found that in the U. P. Terai out of every 100 marriages among the indigenous population, only about 6 were sterile, as compared with 16·8 among the more susceptible immigrant people.

Brahmachari (1923) investigated the vital occurrences during a period of 5 years in three adjacent localities in Bengal. He states that in the Jangipur circle, where malaria had then a comparatively low incidence, the conception rate per mille of population per annum was 47·21; in the Nimitia circle where malaria was declining rapidly from a high level, it was 54·37, while in the Bokhara circle where the disease was very severe it was 41·20. This worker also notes that malaria 'is notorious even to the knowledge of the lay villagers in bringing down the fertility of the affected population'.

In French Guiana, where malaria is very severe, Orgeas (1883) reported that of 418 marriages of French women, 215 were sterile.

The observations quoted above point definitely to a distinct action of malarial infection in affecting deleteriously the conception rate and thus the birth rate.

(b) EFFECTS OF MALARIA UPON THE DURATION AND COMPLETION OF PREGNANCY.

It has long been recognised that malaria is responsible for a large number of interrupted pregnancies*.

Christophers (1911) remarks upon the great increase of abortions, premature labours and still-births during the Punjab epidemic of 1908. Gill (1928) also states that the grave forms of malaria seen at such times are responsible for large numbers of this type of accident. In Amritsar during the epidemic of 1908, the number of recorded still-births in the last four months of the year, when the sickness was at its height, was 586, a number which was nearly 5 times the normal. 'The effect of epidemic malaria upon the fecundity of the population of Amritsar in the autumn of 1908, a city containing in September 1908, approximately 4,600 pregnant women, was to occasion nearly 300 premature births and 1,100 still-births, miscarriages and abortions', i.e., 30 per cent of interrupted pregnancies.

These disasters are not confined to times of epidemics, but occur in places where malaria is only endemic.

* *Vide* footnote on page 245.

Orgeas (1883) states that among the European women of French Guiana, miscarriages were very frequent because of malaria, and that their number was at least equal to that of normal births. Wise (1920) records this disease as being the cause of increased frequency of abortion in British Guiana. Conti (1910) notes that abortion and miscarriage, which he attributes to malaria, are very common in Sardinia. Nagger (quoted by Anderson, 1927), who observed women in the Egyptian oases, records the frequency of abortion, premature labour, precipitate labour, still-births and puerperal hæmorrhage. In only a minority of malarious women was labour and the puerperium normal. Vignes (1922) reports that pregnancy often tends to light up latent malarial infection, and severe attacks of the disease may follow. These frequently lead to premature delivery and grave symptoms during the puerperium. Thomson (1935) remarks upon the effects of malignant tertian malaria in causing abortions and still-births among the natives of Nyasaland. He also notes that acute clinical malaria may show a rapid onset after the birth of the child. This is especially the case in European mothers, in whom the occurrence of fatal coma and blackwater fever after childbirth must be considered.

It was pointed out by Bentley (1911) that in Bombay the proportion of still to live births was raised in the malarious parts of the city, and more especially during those years when the disease was most markedly prevalent. Robertson (1909) in the U. P. Terai noted the frequent occurrence of premature births to malarious mothers, and this was also reported by Newell (1913) in the Punjab. While Watson (1924) states that in the malarious parts of Assam, the women of the immigrant population rarely become pregnant and if they do they usually abort.

Apart from such general statements several workers have given figures in relation to the frequency of interruptions of pregnancy in malarious women.

Weatherley (1895), in speaking of malaria, found in England that in proportion to 56 confinements at term there were only 2 abortions or 3.56 per cent. In the healthy parts of Africa the figures were 35 and 2, or 5.71 per cent, while in the unhealthy parts of that continent there were 20 abortions to 40 confinements, or 50 per cent. In Calcutta, Weatherley found 28 abortions to 60 confinements, or 46.6 per cent. Pellereau in Mauritius reports very similar findings to the Indian ones.

Dyson (1911) states that in the 'wet' areas of the province of Bombay, the percentage of still to living births varies from 15 in December and January (the malaria season) to 2 or 3 in June. On the other hand in the 'dry' areas, where malaria is much less prevalent, this percentage rarely exceeds 2 or may fall below one.

Bentley (1909) has drawn attention to the losses due to miscarriages among the population of the tea gardens in the Bengal Duars. He states that the proportion of miscarriages to full-time births is from 50 to 70 per cent, as against 3 to 5 per cent in non-malarious countries. Das (1923) estimated that by abortion the potential population of Bengal is lessened annually by 800,000 persons, and he judged from his experience of 185 cases that 37.8 per cent of these occurrences were caused by malaria.

Brahmachari (1923) investigated carefully for 5 years the vital occurrences in three adjacent circles in the Murshidabad district of Bengal, *viz.*, Jangipur, Nimtita and Bokhara. The incidence of malaria 'was very low at Jangipur. In Nimtita, it was coming downhill (post-epidemic). But the villages of Bokhara, which had hitherto been healthy, were in the clutches for the first time', and were 'the scene of the disease at the early and most destructive stage of its endemicity'. In Jangipur the percentage of still-births to conceptions during the 5 years was 2.7, in Nimtita it was 3.1, while in the very

malarious Bokhara circle it was 5·7. 'The very high rate in Bokhara is no doubt due to malaria raging with the destructiveness which is usual to it at the onslaught among a population that had hitherto been healthy'.

Similar observations have been made in other parts of the world. Laffont (1912) records abortion in 8 per cent, and premature labour in 28 per cent of malarious women. Laffont and Jahier (1930) report that malaria in Algeria frequently determines the interruption of pregnancy. They found that this disease was responsible for 14·2 per cent of abortions and 10 per cent of premature labours in malarious mothers. Di Pace (1924) investigated the relation of malaria to interrupted pregnancy in Italy. He records that of 68 pregnant women with mild malaria and not receiving treatment, 9·7 per cent aborted, 33 per cent had premature labour, and 20 per cent of the children were born dead. Of 57 women with severe malaria and untreated, 12·5 per cent aborted, 35 to 50 per cent had premature labour and 40 to 66·5 per cent had still-births. In untreated cases of chronic relapsing malaria, 16 per cent aborted, 40 to 47 per cent had premature labour, and still-births occurred in 50 to 100 per cent.

It is evident from the observations recorded above that malaria, both in its epidemic and endemic forms, has a very marked effect in interrupting pregnancy, and also upon the life of the child *in utero*. The loss which is caused by this action must have a very marked effect upon the natural rate of increase of any community, which is afflicted by this disease.

Apart from the effects of these accidents upon the natural increase of the population, one cannot humanely shut one's eyes to the sorrow of the parents who have been robbed of children, nor to the suffering and ill health caused to the mothers by abortions, miscarriages, etc. Here, also, the frustration of the maternal instinct is a fact which cannot be overlooked, more especially in India where the ambition of all wives is to have children, and particularly male children.

(c) CONCLUSIONS.

There is very much evidence to prove that malaria, both in its endemic and epidemic forms, has a very marked influence in lowering the birth rate of any population in which it is prevalent.

The disease acts on the birth rate by (a) reducing the number of conceptions, and by (b) causing interruptions in pregnancy which results in abortions and still-births.

Malaria is, therefore, an important direct factor in checking the natural increase of any population which is afflicted by it.

(II) THE EFFECTS OF MALARIA UPON THE DEATH RATE.

'If the sword has slain its thousands, the malaria has slain its tens of thousands..... This is the Destroying Angel, the pestilence which walks at noonday; and to which all the other causes of mortality are but as feeble auxiliaries in the work of destruction' (Macculloch, 1827).

'If a census were taken among the world's workers on disease, the judgment to be based upon damage to health and direct mortality, the votes would be given to malaria as the greatest single destroyer of the human race' (Osler).

'Manson declares that malaria causes more deaths, and more predisposition to death by inducing cachectic states predisposing to other affections, than all the other parasites affecting mankind together' (Howard, 1909).

'The hecatomb in tropical climates is incalculable' (Le Dantec, 1924).

'It has been stated that malaria has probably killed more human beings than all the wars that have ever devastated the earth' (Current Comment, *Jl. Amer. Med. Assoc.*, 1924).

'The loss occasioned by mortality due to malarial fevers is one of the most serious evils affecting the health and happiness of the people' (Second Pan-American Scientific Congress, *quoted by Hoffman, 1928*).

The serious light in which the medical and scientific world regard the damage done to the peoples of the world by the mortality from malaria is reflected in the quotations given. Howard (1909) says that Creighton has estimated that this disease produces half the mortality of the human race, while other workers assert that it kills more people in one year than the Great War did in five.

Within the bounds of the British Empire are situated a very large proportion of the malarious tracts of the world, and the direct loss of lives from this disease must be incredible. The Health Organisation of the League of Nations (1932) has attempted to obtain some statistics as to the malarial mortality in different countries. The following are some of the mortality rates per 100,000 of the population reported from, or estimated in, different parts of the British Empire :—

Ceylon, 32·6; British Guiana, 350—560; British Honduras, 598·7; British India, 500; Jamaica, 34·5; Mauritius, 639; Straits Settlements, 433.

It is very difficult to calculate with any degree of accuracy the mortality caused by malaria. This is more so because the disease is most common among the less civilised, and less organised portions of the population.

As has been remarked by Christophers (1924), 'it is much to be deplored that we know so little about this important aspect of malaria'. However, statistical enquiries made in various parts of India afford data from which some idea can be obtained about the ravages caused by this disease, but any figures quoted can only be in the nature of estimates.

In any consideration of this subject one must take into account, not only the deaths directly due to malaria, but also those in which the debility caused by the infection predisposed to other affections, which eventually resulted in the death of the individual.

(a) DIRECT EFFECTS OF MALARIA UPON THE DEATH RATE.

'That malaria is the most important cause of mortality in India is scarcely to be doubted' (Christophers, 1924).

'Having regard to the terrible mortality arising from malaria and the wide area it covers (in India), I think, I am entitled to hold that it has become a grave national problem' (Banerjee, 1916).

'The toll of life exacted in India every year by epidemic diseases is still very high, and of them all malaria is perhaps the most devastating' (Report of the Royal Commission on Labour in India, 1931).

Malaria is pre-eminently a disease of tropical and sub-tropical climates, and at least one-third, possibly one-half, of the world's population in such malarious areas is concentrated in the Indian Empire. In view of the mortality caused by this disease, the problem in India is most serious.

Ross (1911) states 'in India alone it has been officially estimated to cause a mean annual mortality of five per thousand; that is, to kill every year on the average 1,130,000 persons—a population equal to that of a great city'. 'This is more than the mortality of plague at its height or of cholera and dysentery combined'. If this calculation be accepted, the present mortality in the whole of India, based on the census of 1931, would be about 1,764,000 persons per annum, or, in British India alone, 1,350,000. Sir Nasarvanji Choksy (1933) in his address to the Council of State in India thinks that 'one out of every 3.5 deaths in India may fairly be assigned to this cause alone'. On the latter estimate the deaths during 1931, a year which was not specially abnormal in regard to malarial incidence, would amount to about 1,900,000 in British India alone.

In the light of these statements, it would appear that the deaths due directly to malaria may be anything from one to two million per annum in an ordinary year. These seem incredible numbers and, if shown to be even approximately correct, indicate the very grave nature of the problem. It is therefore necessary to consider the evidence available to support such views.

The Public Health Commissioner with the Government of India in his Annual Report for 1931 tabulates some of the death rates from malaria which have been recorded in different provinces in India. He, however, points out the imperfect nature of these figures and that separate malaria statistics, even for what they are worth, are not available for all areas in India.

The following are the main malarial mortality statistics collected by him:—

The rates may conveniently be classified as (i) *percentage of total mortality*—Bengal, 31.3; United Provinces, 71.4*; Bombay, 4†; and Coorg, 60. (ii) *Rate per mille of population*—Bengal, 7; United Provinces, 19.3; Bombay, 1†; Burma (urban population), 1.3; Coorg, 14.3. (iii) *Percentage of 'fever' mortality*—Bengal, 47.7; United Provinces, 91*; Bombay, 11†; Burma (urban population), 38; Coorg, 80.

• These figures show *reported* malaria death rates ranging from 1 per mille in the Bombay Province to 14.3 in Coorg, and 19.3 in the United Provinces. This is a very wide range, even when one takes into consideration the great variations in the intensity of malarious conditions in the different provinces. These extreme variations in recorded mortality rates probably depend to a large extent upon imperfect data. The figures are, therefore, mainly of value as an indication of the great prevalence of the disease, rather than a basis upon which any accurate estimate can be compiled.

Christophers (1912) in the Andaman Islands, from the post-mortem examination of convicts who died in hospital, reports that 10–14 per cent died of malaria at that time.

The sub-continent of India is so large that the climatic and physiographical conditions conducive to malaria vary very markedly from place to place, and

* The Director of Public Health of these provinces considers these figures to be excessive, and believes that about 20 per cent. of the fever mortality may be accepted as nearer the truth.

† These figures seem exceedingly low, if one takes the malarial mortality figure of 2.83 per mille given in the Report of the Royal Agricultural Commission to India in 1928 for the province, and rates of about 15 in certain parts of Sind. Even in Bombay City, which cannot be taken as equal to an average of the incidence of malaria in the Presidency, Nerurkar (1930) gives a death rate of 1.4 per mille from 'malaria' and 'ague'.

with these the malarial mortality*. In some parts of India the malarial mortality remains at a more or less constant level throughout the year, except for slight rises at certain seasons. In some of these localities, where the endemicity of the disease is low, malaria accounts directly for a comparatively small proportion of the total mortality. On the other hand, in areas of hyper-endemicity it may play a major part in determining this mortality. The deaths from malaria in the localities where it is endemic are a constant drain upon the population, and in the aggregate mount up to enormous numbers each year. This wastage is so insidious that it has in many places come to be looked upon as a normal state of affairs.

There are, however, large areas of India, more especially in the north and west, in which the mortality from endemic malaria is comparatively low. In these parts there is usually a distinct rise in malarial incidence and mortality in the autumn of each year. In ordinary years this rise may be slight, but such areas are liable to periodical exacerbations of this autumnal rise, which may assume epidemic form. These 'fulminant' or 'regional' epidemics may be responsible for a very heavy mortality among the population of the tracts affected. The death rate assumes proportions resembling those caused by other epidemic diseases and gives rise to much alarm, both to the lay public and to the authorities. Although the mortality at such times may be enormous, the length of the intervals between the epidemics, probably makes them responsible directly for fewer deaths in the long run than the more insidious and continual ravages of endemic malaria.

It is, therefore, necessary to consider the mortality due to endemic and epidemic malaria separately.

(1) THE DIRECT MORTALITY DUE TO ENDEMIC MALARIA.

Estimates of the probable mortality directly due to malaria have been made from the deaths reported as due to 'fevers', the chief cause of recorded mortality in India. 'Fevers' were responsible for a death rate of 14.9 per mille of the population of British India in 1931, as against a decennial mean of 15.6 per mille. This equals nearly 60 per cent of the total mortality from all causes, as compared with only about 5 per cent due to the three main epidemic diseases—cholera, plague and smallpox. The total number of deaths recorded in 1931 as due to 'fevers' was in British India 3,956,100, against a mean of 3,783,972 in the previous decade.

Under the term 'fevers' have been registered a large number of deaths from many diseases, whose predominant symptom is fever. Although the malarial group is the largest and most important of these, especially in rural areas, it would be a great fallacy to attribute to malaria all these deaths. 'The latter ("fever" deaths) should not be looked upon as deaths from malaria *plus* certain wrongly diagnosed cases, but as the total number of deaths *minus* a certain proportion of deaths specifically returned under one or other of the few remaining headings given in the returns' (Christophers, Sinton and Covell, 1931).

* An idea of the relative intensity and distribution of this disease may be obtained from a study of the 'Malaria Map' prepared by Christophers and Sinton (1926).

Leslie (1910) stated that, from indications afforded by certain special enquiries into dispensary reports and other records, it has been estimated for the whole country that about one-fourth of the recorded 'fever' deaths in an ordinary year are due to malaria. Lukis (1916) said that not more than one-half the deaths associated with 'fever' are actually caused by malaria, possibly much less. In the Annual Reports of the Public Health Commissioner with the Government of India, this proportion has in some past years been given as about one-third, while Hehir (1927) thinks from one-third to one-fifth would be a fair average. The Director of Public Health of the United Provinces in 1931 considered that about one-fifth of the 'fever' deaths in those provinces were due to malaria. As will be seen from the figures quoted by the Public Health Commissioner with the Government of India (p. 238), the percentages reported in the different provinces in 1931 show marked variations between 11 and 80 per cent.

Fry (1914), from his investigations in Bengal, says that 'the result was to indicate that two-thirds of the deaths reported under the "fever" heading and classed as malaria had no connection with that disease'.

Christophers, Sinton and Covell (1931) remark that 'it has been computed from the study of vital statistics in various parts of India that, of the total deaths attributed to "fevers", only from 30 to 50 per cent are actually due to malaria. The percentage is probably higher during epidemics and in hyper-endemic areas'.

Several investigations have been carried out in different parts of India, in attempts to determine what proportions of the reported 'fever' deaths were probably due to malaria directly. The results of many of these enquiries are summarised in Table II. These figures were originally collected by careful enquiry from the relatives of large numbers of deceased persons, as to the symptoms, etc., associated with death in these cases.

It appears from the results tabulated that the proportion of 'fever' deaths considered to be malarial varies considerably. The figures, very approximate as they are, suggest, however, that there is some degree of correlation between this proportion and the amount of malaria present, as judged by the splenic index. In hyperendemic areas (splenic rate 60 to 80 per cent), malaria appears to account for from 40 to 50 per cent of the 'fever' mortality. In areas of high endemicity (splenic rate between 25 and 50 per cent), the proportion is about 30 per cent, while in areas of low endemicity the figures may be about 10 per cent.

The places in which such a small proportion as 10 per cent has been reported, have been towns where the malarial incidence is low. It is well known that urban areas suffer much less severely from this disease than do rural ones, and the case mortality from malaria is probably less, because of the greater facilities for treatment in towns. Only about 1/20th of the total mortality in India in 1931 was reported from urban areas, although about 1/10th of the population of the country dwell in towns. It is obvious from these figures that so low a figure as 10 per cent of the fever deaths cannot be taken as representative of the average death rate from malaria in British India as a whole.

The returns for 1931 show that the fever death rate in rural areas is twice that of towns, so it appears possible that the malaria death rate in villages is

TABLE II.
Verified malaria fever and total mortalities.

Locality	Authorities.	Splenic index.	Fever rates per mille of population.	Percentage of fever deaths due to malaria.	Malaria mortality per mille.	Percentage of total mortality due to malaria.	Number of deaths investigated.*	Total mortality all causes per mille.	REMARKS.
Dinajpur district (1904)	Rogers (1910) (Bentley, 1916).	28-95 av. 50	..	31·8	..	27·0	1,104 (F)	..	
Dinajpur town (1909-1910).	Gol quoted by Bentley (1913a, 1916).	27·0	16·5	33·3	55·†	21·0	317 (F)	267	† Bentley (1916) estimates 73.
Jessore, Nadia and Murshidabad districts.	Stewart and Proctor (1907).	55·9	31·8	36·1	11·5	34·5	835 (F)	39·6.	
Keraniganj Thana, Dacca district.	Bentley (1913)	6·8	18·0	28·15	5·0	15·7	4,304 (F)	32·2	Total.
Burdwan district and 24-Perganas.	Bentley (1916)	25·0	17	5·0	..	33·7	
Burdwan district (1906)	Reported by Fry (1912).	12-40	7·9	26·0	..	30·6	
Dum Dum	Ghosh in Fry (1912).	50-50	..	51·45†	4,859 (F)	..	† Directly and indirectly.
Cossipore-Chitapore (1907-1911).	Brahmachari (1913).	11·5	16·37	34·7	..	24·0	219 (F)	..	
Central Provinces	Kenrick (1914)	80	28·3	12·4	1·6	5·3	6,320 (A)	32·2	
Terai of U. P.	Robertson (1909)	74-88	29·9	47·0	13·3	35·0	333 (F)	38·0	
				42·0	14·0	35·6 (47·3)§	562 (A)	32·63	§ Excluding cholera, plague and smallpox.
Nagina, U. P.	Robertson (1910)	79·1	28·06	44·9	12·6	32·9	485 (A)	35·22	
Lucknow City	Graham (1915)	2·2	23·0	10·6	2·4	3·8	556 (F)	46·8	Malarial season.
		4·6	1·06	1·4	197 (F)	..	Non-malarial season.

* 'F' = deaths from 'fever'; 'A' = deaths from all causes.

more than twice that in towns. This idea is supported by the figures given in Table II.

From the evidence available, one appears justified in considering 25 per cent of the 'fever' deaths, as a *minimal* estimate of the average proportion of such deaths due *directly* to malarial infection. If this proportion be taken, one finds that in a year of ordinary malarial prevalence, such as 1931, about one million persons died in British India alone as the *direct* result of this disease.

During the months from February to July inclusive, *i.e.*, the months when malaria is least active, the total number of fever deaths recorded in British India was 1,782,283. From the data given above, probably at least 1/10th of these were due to malaria, that is about 178,000. During the malarial months of the year, August to January, 2,173,813 deaths were reported. If we consider one-third of these deaths during the malarious months, a low estimate, as being caused by this disease, the number would be about 724,000. The combined total for the year would then be over 900,000 malarial deaths, or about 3.3 per mille of population.

Leslie (1909) suggested that in an ordinary year malaria was responsible for a mean death rate of 5 per mille. The investigations summarised in Table II show an estimated death rate between 2 and 14 per mille, varying with the intensity of the malarial incidence as judged by the spleen rates.

In Bombay, Nerurkar (1930) records a death rate of 1.4 per mille from 'malarial fever, ague and remittent fever', while Majumdar (1927) gives the malarial death rate in Calcutta as 1.6 per mille. These may perhaps be taken as minimal death rates in areas of low endemicity, for in neither of these cities is the severe type of malaria, relatively speaking, very prevalent, and the facilities for treatment make the case mortality very much less than that of rural areas where the disease is more widespread. In Meerut, Graham (1913) investigated the malarial death rate and reports it to be about 4 per mille.

Taking these urban rates into consideration, and remembering that about 90 per cent of the population reside in rural areas, where malaria is most prevalent, Leslie's estimate of a death rate of 5 per mille of the population seems a moderate one, as representative of the average rate for British India as a whole. Even if this rate be accepted, the deaths in British India, directly due to malaria, would be about 1,350,000 per annum.

Summary

From the evidence available, it appears that at a minimal estimate at least 1,000,000 persons die each year in British India, as the *direct* result of endemic malaria. It is also very probable that such an estimate is much below the true figure.

(2) MORTALITY FROM EPIDEMIC MALARIA.

If the vital statistics of this country be examined, it will be seen that from year to year there is a distinct rise in the total mortality rates all over the country, during those months of the year when malaria is most prevalent. This rise, due to an increase in 'fever' mortality, is usually so constant that it may have little effect in producing any variation in the total mortality rates from year to year.

Such seasonal rises, affecting both mortality and morbidity, are usually most evident during the autumn months in those places where the amount of endemic malaria is comparatively low. The areas which show these variations most markedly are more common in the northern and western parts of India. The mortality from the 'normal' autumnal outbreak of 'fever', in even an ordinary year in the Punjab, may cause large numbers of deaths in the areas of low endemicity. This is shown by the fact that, although in this province during 1931, there was no widespread or severe epidemic of malaria, yet it was calculated that malaria caused 30,000 deaths, *i.e.*, an increase in mortality equal to 1·3 per mille of the population.

Though the increased mortality in normal years may be relatively low, it may be as much as 25 per cent in others. Every few years there may also occur severe outbreaks of malaria, resulting in an enormous increase in mortality over greater or lesser portions of the affected tracts. Under these conditions the number of deaths per mille in a single month may be equal to the annual death rate for a whole year in Great Britain. The mortality caused by such 'fulminant' or 'regional' epidemics requires special consideration. They are most common in the Punjab and Sind, but may extend into adjacent provinces. Their distribution is indicated in the 'Malaria Map' prepared by Christophers and Sinton (1926).

The degree in which ordinary autumnal or fulminant malaria prevails in any given year is best shown by the 'epidemic figure'*. This figure indicates the number of times the death rate, at the height of the malaria season, exceeds the average normal figure, taken when epidemic conditions of any disease are absent. 'The provincial epidemic figure merely indicates the general intensity of the disease throughout the province. In a year when a major epidemic occurs, this figure may be as high as 10, but in years in which no epidemic occurs, it does not exceed 1·0; but in many years, however, a mild epidemic of restricted distribution is associated with an epidemic figure of 1·1 to 1·5' (Gill, 1928). The fact that, during the epidemic of 1908, the local epidemic figure in some areas rose as high as 19, shows the excessive local mortality which may sometimes be caused by this disease.

During the epidemics which occurred in the Punjab in 1881, 1887, 1908 and 1917, Gill (1928) states that the epidemic figures for the city of Amritsar were 21·8, 10·4, 13·3 and 16·1 respectively: In this city of about 160,000 inhabitants, for many weeks in the autumn of 1908 the mortality was at the rate of over 200 per mille. More than 10,000 deaths were recorded of which it was calculated that 7,000 were due to malaria (Christophers, 1911a).

It is reported by Christophers (1911a) that, during the 1908 epidemic in the Punjab, an area of about 5,000—6,000 square miles with a population of about 20 million of inhabitants was involved. Among this population a quarter of a million deaths occurred in 3 months. 'Many villages and even whole thanas showed mortality rates during October and November of 300, 400 or even 500 per mille'.

* 'For any comparatively local unit, such as a thana, this is obtained by dividing the total number of deaths recorded during the month when the epidemic has reached its greatest intensity (either October or November), by the number of deaths usually recorded in a month when no epidemic of any disease has occurred. In practice the divisor is best obtained by selecting the months which appear to have been free from special epidemic conditions' (Christophers, Sinton and Covell, 1931).

The actual mortality caused by these epidemics of malaria, directly and indirectly, within a few months is almost inconceivable.

Christophers (1911b) states that while the total monthly mortality in the Punjab is usually about 50,000, yet in October and November 1908, as a result of a malarial epidemic, there were 307,316 deaths recorded. In previous epidemics in the Punjab the mortality during these months was 254,580 in 1900, 283,223 in 1892 and 246,487 in 1890.

It is reported by White (1909) that, from September to December, the 'fever' mortality in the United Provinces caused by the epidemic of malaria in 1908 was 1,141,079, or 23.92 per mille of the population, as compared with an average of 511,021 for the previous quinquennium. In an earlier epidemic in these provinces in 1879 the mortality from 'fevers' was 1,140,247, equivalent to 26.9 per mille of the population.

Is this excessive mortality entirely or mainly due to malaria ?

Gill (1917) in his careful study of the effects of malaria in Amritsar states — 'It has been shown that the average "fever" death rate is rather more than half the average total death rate, whilst it is certain that this is an over-estimate of the true malaria death rate, yet it is not open to doubt that the autumnal "fever" mortality, particularly in years when the "fever" mortality is exceptionally high, is almost entirely the result of malaria'. Christophers (1911a) reached a somewhat similar conclusion.

Epidemics of a more localised nature are occasionally reported from other parts of India. Horne (1913a) remarks on the heavy mortality in one such epidemic in the Cuddapah district of Madras, while the Public Health Commissioner with the Government of India in his Annual Report for 1930 mentions an epidemic in the coastal parts of the Ganjam district, in which 30 to 40 per cent of the residents died in some villages.

The suddenness and severity of these epidemics are so striking that they receive wide publicity in the lay press. Although the immediate mortality is much larger than that caused by even severe endemic malaria, yet the unceasing nature of the latter is probably responsible for a greater number of direct deaths in the same number of years.

Apart from such regional epidemics or pandemics, more localised epidemics are constantly occurring. These are usually associated with 'tropical aggregation of labour' in connection with large industrial or agricultural enterprises, and with conditions of economic stress. Such epidemics may be a very serious factor in hindering the economic development of many parts of India (Christophers and Bentley, 1909; Sinton, 1933). Examples of such epidemics in relation to railway construction are given by Clemesha (1917) and by Senior-White and Newman (1932). The mortality which occurs during the construction of railways in highly malarious areas in different parts of the world is notorious, and has given rise to the saying that such work costs 'a life a sleeper'. Clyde (1931) gives a description of a similar outbreak in connection with the construction of the head-works of the Sarda Canal in the Terai of the United Provinces.

Summary.

Epidemics of malaria may cause a great increase in the local mortality in the areas where they occur. When, however, one of the great regional or fulminant epidemics is present in any year, the total mortality of the country may be increased by one-quarter to half a million deaths.

(3) OTHER FACTORS INFLUENCING THE DEATH RATE IN MALARIA.

While sex has not a very marked influence upon the death rate from malaria, this rate is closely related to the age of the persons afflicted and also to the economic conditions under which they live.

(i) *Influence of sex on the morbidity and mortality from malaria.*

Sex *per se* does not appear to have much effect in determining differences in the malarial mortality rates in India. The influence of malaria upon the health of pregnant women, however, requires special consideration. As will be discussed later, malaria in women in this condition may have a very marked indirect influence upon neo-natal and infantile mortality.

There is a superstition common in very many countries that, if quinine be given to a pregnant woman, she is liable to abort or miscarry. For this reason, many pregnant women suffering from malaria receive no specific treatment for the disease until they are *in extremis*, and the dreaded interruption of pregnancy has commenced or taken place*. Untreated infections in pregnant women often develop into pernicious attacks, and the sufferers are, in many instances, brought for treatment too late for their lives to be saved. Among the uneducated, and even among the educated, this superstition has been responsible for the sacrifice of many useful lives.

Laffont and Jahier (1930), among other workers, have noted that malaria in pregnant women makes for frequent cases of anæmia, cachexia and pernicious attacks of this disease. Parturition is also very liable to cause relapses of chronic malarial infections, and Thomson (1931) remarks upon the frequency of these occurrences in some parts of India. Such relapses may be of a very severe type, and may be accompanied by hyperpyrexia and toxæmia leading to a fatal termination.

During the puerperium, apart from pernicious attacks and severe relapses, uterine hæmorrhages and delayed involution of the uterus are not uncommon. If the woman survives the combined effects of malaria and parturition, the resultant debility renders her more liable to contract some intercurrent affection, which may carry her off at a later date.

(ii) *Influence of age on the mortality in malaria.*

The age at which the chief mortality from malaria occurs has an important relation to the financial losses which this disease causes to a locality or a country. The earlier the age at which such deaths take place, the less opportunity have the victims had of contributing to either the natural increase of the population or to the wealth of the country. Apart from the fact that persons dying below

*It has been shown clearly by many reliable workers that quinine has comparatively little or no effect upon the *quiescent* uterus, and that the interruptions of pregnancy reported in malarious women are almost without exception due primarily to the disease and *not* the remedy. Interruptions of pregnancy occur in untreated and tardily treated cases of malaria, in which the disease has started a contraction of the uterus. If the woman had been properly treated for her disease at an early date, the chances of a disaster to pregnancy would probably have been averted, at least in the majority of cases.

the age of 10 years have contributed little or no material return to the community, the cost of their feeding, education, etc., is an item on the debit side of the account.

While it is generally accepted that, in India and in other malarious countries, the direct mortality from malaria falls most heavily upon the infants and children of the afflicted population, it is not easy to obtain any precise figures in the matter. Various workers have investigated the age incidence of deaths due to malaria in endemic areas. As the majority of such deaths occur among individuals who have never been seen by a qualified medical practitioner, and as post-mortems are usually unobtainable under ordinary conditions, the figures have been based mainly upon the results of enquiries made from relatives as to the signs and symptoms of the ailment which preceded death. The findings recorded by some workers in India are given in Table III.

TABLE III.

Age in relation to mortality from endemic and hyperendemic malaria.

Locality	Authority.	PERCENTAGE OF MALARIAL MORTALITY OCCURRING IN AGE PERIODS (YEARS)—									
		Under 1	1-5	6-10	11-15	16-20	21-30	31-40	41-50	51-60	Over 60
Dinajpur	Rogers (1910).	75									
Nadia, Jessore and Murshidabad.	Stewart and Proctor (1909).	4.9	28.7	14.9	6.6	6.0	10.6	11.5	8.2	5.6	2.6
U. P. Terai	Robertson (1909).	15.7	26.8	7.4	6.0	7.4	11.5	12.5	4.6	4.1	3.7
Nagina	Robertson (1910).	26.2	24.4	6.9	8.7		15.0		12.5		6.2
Kairana	Graham (1910a).	28.4	22.0	6.4	10.0		19.3		13.8		..
Kosi	Graham (1910b).	16.6	16.6	8.3	8.3		41.6		8.3		..
Meerut	Graham (1913).	35.6	26.0	7.0	6.4		11.7		12.0		1.3
Lucknow	Graham (1915).	15.2	30.5	5.1	6.8		28.8		8.5		5.1

From these records, it would appear that probably about 35 to 60 per cent of the deaths directly due to malaria occur during the first 5 years of life, and that the majority of this is between the ages of 1 and 5 years. From 40 to about 70 per cent of the total malarial death rate in endemic areas is recorded during the first 10 years. One would expect this because (a) children have probably not so much natural resistance to the effects of malaria as have adults, and (b) the majority of the adults, who have survived the dangers of malarial infection in childhood, have probably acquired a considerable degree of immunity to the more serious pathogenic effects of the local strains of parasite. That these factors have a great effect, is supported by the fact that the mortality rate falls markedly after the 5th year of life.

Bentley (1925) says that this destruction of child life is reflected in the proportion of children under 10 years of age in different malarious localities. This is illustrated in Table I.

Most observers are agreed that the direct mortality from malaria is heaviest during the early years of life. The fact that in certain hyperendemic areas the child population may seem fairly healthy, has made some workers doubt whether this disease is such a great cause of infantile and child mortality as was formerly supposed to be the case. This point requires further investigation*.

It will be seen from a study of Table III that the direct malarial mortality among adults is relatively much less than among infants and children. Robertson (1909) found that deaths from chronic malaria were fairly evenly spread throughout the different age periods, with perhaps some rise at the 30–40 year period. Kenrick (1911) considered, as the result of his enquiry in the Central Provinces, that mortality among adults was much more commonly due to the indirect action of malaria than to a direct one. Intercurrent diseases carried off the individuals weakened by the continued ravages of chronic malaria.

Stewart and Proctor (1907) report that they found, among 291 'fever' deaths in children under 10 years of age, 30·6 per cent were due to acute malaria, and 19·9 per cent to chronic manifestations. Of 440 'fever' deaths among adults, 13·6 per cent were due to acute and 12·9 per cent to chronic malaria. 'Therefore, while the proportion of "fever" deaths due to malaria is greater in children than in adults, still there is a large mortality among adults directly from malaria, and that this is so must be attributed solely to the fact that they

* This apparent discrepancy may depend to some extent upon the fact that, in many of these hyperendemic areas, the child population often contains a large proportion of children of aboriginal parents, who are well known to be very prolific. In such races there is evidence to support the view that a considerable amount of natural or rapidly acquired tolerance to malaria is present (*vide* footnote, p. 232). However, Robertson (1909) and Graham (1913b), in their investigations into malaria in the Terai of the United Provinces, remark on the high infantile mortality due to malaria among such aboriginal races, but that this is less than among the immigrant people of Aryan origin. Phillips (1925) also points out that the average annual infantile mortality among the Bhuksas (aborigines) was 401·9 per mille, as compared with 533·7 among the Desis (immigrants). This worker paints a graphic picture of the cheerful and energetic character of those Bhuksa children who survive, as compared with the pale anæmic and weakly Desis. Among the African negroes, Brumpt (1922) quotes Steuber as saying that, in East Africa, 50 per cent of native children die before the age of 4 years, mostly from malaria. Thomson (1935), in Nyasaland, also emphasises the very high infantile mortality, which he thinks may be largely due to malaria.

It will be necessary, therefore, in future investigations into the effects of malaria upon the mortality of the child population, to draw a clear distinction between these two different types of races, before any conclusions are drawn.

do not get sufficient treatment with quinine, as among persons properly treated malaria is not a fatal disease except in a small proportion of cases'.

As in endemic malaria, the mortality during epidemic outbreaks falls mainly upon the infant and child populations. This fact has been very clearly shown by Christophers (1910, 1911a) and by Gill (1917, 1928) in their careful investigations into the effects of fulminant malaria in the Punjab. These workers found that 50 to 70 per cent of the total mortality during such epidemics occurs in the first 5 years of life, and the majority during the early portion of this period.

Christophers and Gill studied the relation of total mortality during epidemics with that seen in non-epidemic periods. Christophers (1911a) divided the epidemic death rate at each age period by the normal death rate for the same age period. By this method he showed that the normal curve of mortality was exaggerated at the two extremes of life. In Amritsar, during the 1908 epidemic, there was a great relative increase in the death rate at about 2 years of age, which lasted until about the age of 10. Deaths under 1 year were twice the normal, at 5 years 8·7 times normal, and in the age period 6—10 years 5 times normal.

Gill (1928) compared the death rates in this epidemic with the average death rates for the decade 1901—1911, and found that there was a 30 per cent increase in the mortality among the children under 1 year, 70 per cent in the age period 1—5 years, and 16 per cent from 5 to 10 years. The latter worker studied carefully another epidemic at Bhera in 1921, in this the percentage increases in these age periods were 137, 241 and 154 respectively. He remarks that regional epidemics are characterised by special liability to attack extreme youth, and enhance the death rate up to 10 years of age. The rate is relatively high in the first year of life, declines rapidly up to 5 years, and then gradually up to 10. The rise in the proportion of infant and child deaths to total deaths at the outbreak of an epidemic, was also noted by Kenrick (1914) in the Central Provinces.

White (1909) states that, in the United Provinces, after the epidemic of 1908 'the Civil Surgeon, Muttra, informs me that in some villages 75 per cent of the children died, and there were no children left to vaccinate'. Horne (1913a) records a similar increase of mortality among children during an epidemic in the Cuddapah District of Madras. In this the infantile mortality rose from an average of 151·6 during the previous 5 years to 232·9. In an epidemic in another area of the same province, in one month the deaths between the ages of 1 and 10 years were increased to 25 times the normal.

Although the morbidity among the adult population during an epidemic may be enormous, yet, while the death rate increases, it does not do so in anything like the same proportion as seen among children. As pointed out by Christophers (1911a), the curve of the death rate is an exaggeration of the normal one, in which the curve is highest at the two extremes of life. Some figures given by this worker as to the age incidence of mortality during a malarial epidemic in the Punjab are shown in Table IV. These show the much smaller proportion of deaths which occur during early adult life and middle age. Horne (1913a) reports that in an epidemic in the Madras Presidency the death rate among children (1—10 years) was 25 times the normal in December, while at each decade from 20 to 60 years it was only 3 or 4 times.

TABLE IV.

Age incidence of mortality during an epidemic of malaria in the Punjab.*

Locality.	PERCENTAGES OF TOTAL MORTALITY OCCURRING DURING THE AGE PERIODS—									
	Under 1	1-5	6-10	11-15	16-20	21-30	31-40	41-50	51-60	Over 60
Amritsar	14·4	32·8	8·3	3·2	2·3	5·3	4·2	5·3	6·3	18·2
Bhera	35·0	33·0	6·9	3·2		2·4	1·9	1·2	3·2	12·7
Palwal	55·7		8·5							

* Compiled from figures given by Christophers (1911a).

Gill (1917) remarks that in Amritsar a high infantile mortality and a high fever rate (mainly malarial) rise and fall together with great uniformity, except in years when famine and scarcity also increase the former rate. He considers that malaria is largely responsible for the height of the infantile mortality in this city, more especially in the autumn and in those years when malaria is epidemic.

The salient feature of the epidemic conditions is, therefore, its excessive mortality, which is conspicuously marked among infants and young children.

It is evident from the information available that from the period of intra-uterine life up to the age of puberty, malaria is a grave danger, directly and indirectly, to the population of a malarious country. This danger is most marked in the years below 5. Among the children it is necessary to consider separately the mortality among infants before the age of 1 year, and those from this age up to 10 years.

As Ross (1911) has pointed out, probably the great majority of malarial deaths occur among the children of the poor, and in the tropics these are seldom brought to hospital or attended by a medical man. In India a very large proportion of the population, being rural, live at some distance from the Government dispensaries, usually the only source of medical aid in such districts. When children become ill, it means that, if they are to receive medical attention, they have to be brought to the doctor by some adult. Under the economic conditions prevailing in this country, few households can afford the time necessary for adult members to take the children several miles daily to hospital, with the result that large numbers of sick children recover or die without any proper treatment. The medical officer in charge of the dispensary can seldom spare the time to go long distances away from his headquarters, nor can the population afford to pay the fees for such visits. As remarked by Stewart and Proctor (1907) in their report on Bengal, 'the large infantile mortality due to malaria is not surprising when such a large proportion of the infants are shown to have suffered repeatedly from the disease, especially when it is remembered that most of the cases are absolutely untreated'.

It is evident from the many observations quoted above that the direct action of malaria is specially destructive of child life. Many deaths are also caused by its indirect action.

The fatal action of the disease upon the younger generation depends mainly upon the severity of the acute parasitic attack. If the individual survives such attacks, as he grows older the sequence of events is probably that he gradually acquires infections with most, or all, of the local strains of parasite. With clinical recovery from the acute attacks resultant from these infections, he develops a considerable degree of immunity against the clinical effects of re-infection with any of such strains. This immunity or 'tolerance' to clinical effects lasts as long as the individual has a chronic or latent infection with these strains, and such infections are being kept alive by continual vaccination and revaccination through the bites of infective mosquitoes. Under these conditions, the causation of increased morbidity and mortality by re-infection with such strains is greatly reduced. However, severe and dangerous attacks may still be induced in such individuals by various factors such as—

(a) a lowered immunity, possibly as the result of infection with some other disease, or of economic stress; or

(b) a loss of immunity following on parasitic cure, either by natural or therapeutic means; or

(c) an increased dosage of infection (sporozoites), as may occur during epidemics or in hyperendemic areas; or

(d) infection with new strains of parasites, as the result of immigration to a new locality, or the introduction of fresh strains into the local environment; or

(e) a possible exaltation of the virulence of the local strains of parasite from some unknown cause; or

(f) a combination of these factors, as may occur under conditions of 'tropical aggregation of labour'. These points have been discussed by Sinton (1933).

Apart from acute infections predisposed to by the above factors, any increased mortality among the older children and adults appears to be due mainly to the debilitating effects of chronic infections in susceptible or less resistant individuals, whereby they fall victims to secondary diseases predisposed to by this lowered vitality.

During the first 2 or 3 months of life, malarial infection *per se* is probably not a very great *direct* factor in causing infantile mortality. There is fairly conclusive evidence that true 'congenital malaria' is exceptional, and cases reported as such almost always occur as the result of accidents in pregnancy or during labour, whereby the fœtus or infant becomes infected directly from the maternal blood. The infantile deaths resulting from malarial incidence in the population during the early months of life are mainly due to the indirect effects of the disease. These indirect effects are secondary to either debility or immaturity of the child born of a malarious mother, or to sickness or death of the latter. These points will be discussed in greater detail later.

The advent of the period when malarial infection acts directly as a cause of mortality, will depend on the chances that the child has of becoming infected. These will be influenced by the season at which the child is born, and also by

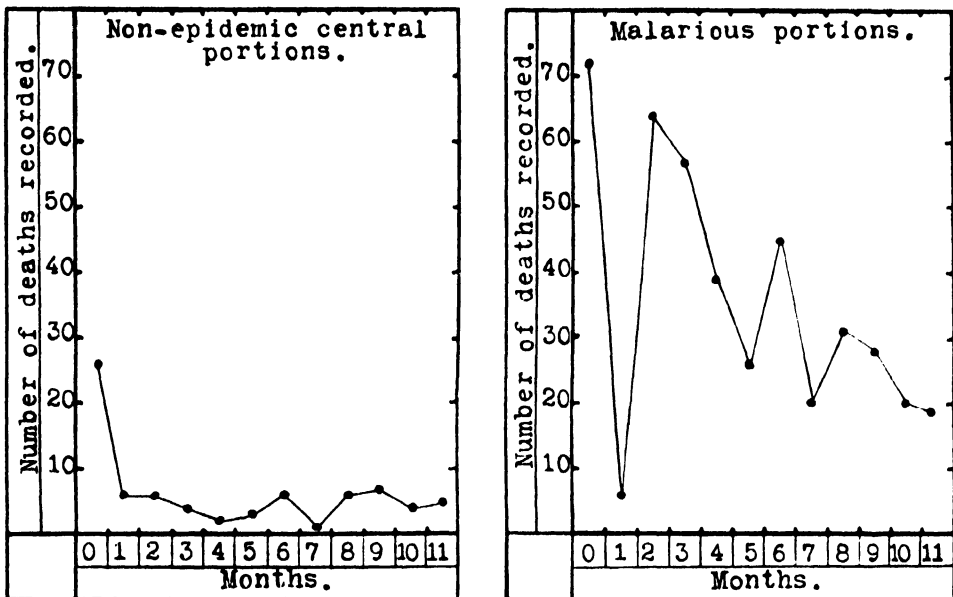
the number of infected mosquitoes which have access to it. Thus a child born in the non-malarious season may escape infection for several months, while one born in the malarious season, especially if the number of infected mosquitoes be great, as in a hyperendemic area or during an epidemic, may very rapidly acquire the infection.

Such a view is supported by some figures given by Christophers (1911a) of the number of deaths occurring among children in different parts of Amritsar during each month of the first year of life. These figures have been shown graphically in Chart I.

CHART I.

Number of deaths recorded during the first year of life at each month of age at Amritsar in the year 1908.

(After Christophers, 1911a)



In this chart the initial high mortality in both areas is probably due to the indirect causes of infantile mortality mentioned above*. In the malarious areas the drop during the next month appears to represent the period between deaths due to these indirect effects and the period when deaths due to acute malarial infections commence†. After the initial rise in malarial mortality, the deaths during the succeeding months probably occur among children who have escaped malarial infection for a longer period, and among the debilitated children who have survived the acute attack and later fallen victims to intercurrent disease or the prolonged action of chronic infection.

* For a fuller discussion of these indirect causes of mortality, see pp. 258-262.

† This interval probably gives some indication of the period necessary for a child to acquire an infection, and develop clinical manifestations leading to death, under the conditions prevailing at the time.

(iii) *Effects of economic stress upon the morbidity and mortality in malaria.*

There is much evidence to suggest that the occurrence of economic stress has a marked influence in determining not only the prevalence of malaria, but also the severity of the manifestations of this disease. These results have a distinct action not only upon the number of deaths from the disease, but also upon the case mortality among those afflicted.

'Floods, poverty and hard times without question are responsible for the following conditions favouring the (malaria) parasite: (1) lowered resistance of the patient; (2) his failure to see a physician; (3) his failure to take quinine or other malaricidal drugs; and (4) last, but not least, increased exposure to infection' (Faust and Diboll, 1934).

Before discussing the influence of economic stress upon the incidence and mortality from malaria, it is necessary to consider what is the economic condition of the people of India under ordinary conditions. This has been summed up in an Editorial in the *Indian Medical Gazette* (1922) as follows—

'Poverty is nowhere to be seen in more acute form than in India. Many millions of the poorer population live on a minimum of food; exist, in what McCollum terms "the twilight zone", where small shifts in the quality of the diet are sufficient to determine a pathogenic state'. 'Under such conditions the play of economic factors in inducing endemic and epidemic disease is bound to make itself felt sooner or later'.

Megaw (1933) also points out that 'periods of famine or scarcity of food have been occurring in one village in five during a ten-year period in which there has been no exceptional failure of rains'.

Bentley has paid special attention to the relation between economic stress and disease. This worker (Bentley, 1913a) states that 'observations among the rural population of Bengal point to the economic condition of village populations as being the main factor determining a moderate or excessive mortality among them. Communities whose members as a whole are well provided with the necessities of life have a mortality rate rather below the average for the province, whereas communities experiencing economic pressure invariably show a much higher death rate. The mortality among village beggars and landless labourers is exceedingly heavy, because they are constantly on the verge of starvation. And villages possessing a large proportion of people of this class show a higher general mortality than others. And among other classes also the occurrence of economic stress is immediately followed by an enhanced mortality. The death of a cultivator who is not in good circumstances and who possesses no adult male relations is almost invariably followed by the speedy death of other members of the family; and the same phenomenon may be observed in the case of petty traders and craftsmen. In this way even among communities that are apparently in a generally prosperous condition, whole families may be decimated, the death of the father being followed by that of all the other members, in an amazingly short space of time'.

The effects of such economic stress upon malarial conditions have been noted by many different observers. Christophers and Bentley (1909) state that Stephens and Christophers have observed a greater prevalence of malaria among low-class communities than among those of better social status in the same locality. Leslie (1910) pointed out that 'in the towns, in India as in other countries, there are numbers of people who lead a hand-to-mouth existence; ill-housed, ill-clad and ill-fed, they pick up a precarious livelihood in the unskilled labour market. Such people have unsuitable food at the best of times, and they have no savings, so that when anything occurs to check the demand for such work as they can do, the scanty coarse food becomes scantier

and coarser, and they and those dependent on them offer little resistance to malarial infection and readily succumb to its effects'.

The various ways in which economic stress may cause, directly and indirectly, an increased malarial prevalence have been discussed at some length by Bentley (1925) who says that—'Physicians know that an acute malarial infection occurring in a person, who is favourably situated as regards food, clothing and housing and who can afford to adopt the proper measures, will usually yield to specific treatment very quickly. It is otherwise with the sufferer from chronic malaria, exposed for a long period to semi-starvation and adverse circumstances of all kinds. In his case specific treatment will fail to achieve its purpose, unless it can be combined with generous diet and other measures for improving his general health and relieving his miserable condition. What holds good for individuals holds good also in the case of communities'.

'No one will dispute the good effects that increased prosperity exerts in reducing both the prevalence and evil results of malaria. Increase the prosperity of a community and at once you enable both the individual members and the community, as a whole, to take more efficient action against malaria. The resistance of the individual to disease is greatly strengthened by good and ample food, better clothing and improved shelter; remedies such as quinine can be used in adequate amount by a prosperous population who can also afford to employ skilled doctors. Special personal preventive measures, such as mosquito nets, also become possible; and profiting by the education which is within their reach, the members of a prosperous community can adopt measures for improving the sanitary condition of their villages in a manner which poverty had previously placed absolutely beyond their reach'.

The same worker (Bentley, 1911) states that, while several different villages were being visited by house-to-house examination, instances were found in which economic stress acted in direct relation to endemic malaria. Frequently the mortality was found to be in one or two houses, so that there might be a village with a total mortality of 20 to 25 per cent the bulk of which occurred in a few families. The families most attacked were those in which the male bread-winner had died during the previous year, or where poverty or a load of debt was present. 'These observations show that economic stress may play an important part in deciding what effect malaria is going to have, whether there is likely to be a heavy mortality or not'.

The combination of economic stress and malaria produces a vicious cycle in which it is often difficult to say which is the most serious factor in the end. Bentley (1913) concluded from his study of rural conditions in Bengal that once depopulation of a village is in progress due to diminished fertility of the soil, malarial infection is intensified. Breeding of mosquitoes increases and a greater degree of infection occurs among the population. These observations point to the economic condition of the village population as being the main factor in determining a moderate or excessive mortality among them. 'This increase of malarial infection, itself produced as the result of depopulation and increasing economic stress acting on a parasite-laden community, tends in the presence of continued economic stress to result in an increasing prevalence of the disease. This again leads to further decrease of population, a further increase of economic stress, a still further intensification of malaria, more and more disease, and so on, until finally the affected population is wiped out of

existence'. The true sequence of events in depopulation in Bengal is according to this worker (Bentley, 1913) 'firstly, a decay of prosperity, and then an increase of mortality and migration, resulting in rapid depopulation, and while this process is going on malarial infection increases in intensity with proportionate rapidity'. 'It was found wherever there was evidence of severe poverty, endemic malaria was intensified' (Bentley, 1911).

Fry (1911, 1912) also noted that certain widespread epidemics of malaria in Bengal had not the characters of true regional epidemics. He considers that they may perhaps be regarded as 'deficiency epidemics', the deficiency being the outcome of economic stress, occasioned by silting of rivers in a previously prosperous tract, in association with partial failure of the monsoon.

The relationship between endemic malaria and economic stress has been noted in several other parts of India. Marjoribanks (1914), in his malaria survey of Salsette Islands, found that the poorer the feeding of a class or caste of people is, the more liable are its members to malaria and *vice versa*. Kenrick (1914) considered that, while scarcity and high prices cannot affect the conditions responsible for the localisation of malaria, yet by lowering the resisting power of the people living in endemic tracts, the malaria rate is increased.

In the Punjab, Newell (1913) remarks that continuous or successive illnesses due to malaria lead to poverty and its results, and so to the upkeep of many diseases. Conditions which lead to insufficient food lessen resistance, and the sufferer may die from malaria or other intercurrent diseases, while the same effects may react upon his dependents.

Gill (1922) studied the heavy malarial incidence in areas of the Punjab where water-logging followed by excessive salinity of the soil is present. He found that, while the introduction of canals may at first bring prosperity, with water-logging prosperity declines, until finally it undergoes an eclipse with increased malaria. This takes the form mainly of chronic malarial cachexia, dependent upon the fact that under extremely adverse conditions the recovery of the infected individuals is retarded and repeated relapses occur. Under such conditions he considers the malarial problem is primarily an economic one. Lal and Shah (1933) studied the same conditions and arrived at similar conclusions. They found the death rate in such villages higher than that of villages in the same locality, but outside the water-logged area. The most conspicuous features in such localities are the poverty-stricken and anæmic appearance of the adults, a paucity of children, high infantile mortality, and a low birth rate.

The great influence of economic stress in increasing the severity of the disease and the mortality from malaria under the adverse conditions of 'tropical aggregation of labour', were clearly described by Christophers and Bentley (1909) :—

'Once sickness, debility and anæmia become rife the pressure and frequency of individual hardship become enormously increased. Pay cannot be earned by the sick, who may suffer actual starvation. Nor is it only the workers who suffer, for by the inability of partially or completely disabled labourers to earn their pay, their relatives and dependents are exposed to greater hardship and increasing liability to sickness; and the greater the number of sick the more intense becomes the general infection, until as a result an immunity that may protect under ordinary conditions is broken down under exposure to more

virulent and intense malaria, so that even those originally the strongest and most healthy become involved also'.

'The orphan with no parents, the man with no wife to do his cooking, the mother without means to nourish her fatherless children, the stranger without friends, and those of poor physique or low intelligence, all suffer under such circumstances to a far greater extent than they would under ordinary rural or urban conditions'.

The correlation between economic stress and the mortality from epidemic malaria, has been studied especially in the Punjab. Christophers (1910) found that in such epidemics the heaviest mortality was among those classes which are poorest and living in the greatest squalor. Particular areas subject to adversity are likely to be picked out, if the other factors be favourable for epidemic conditions. This author (Christophers, 1911a) also showed that the spleen rate after an epidemic was invariably higher among the poorer sections of the community.

The conclusions arrived at by Christophers (1911a) were that the mortality caused by malarial epidemics in the Punjab is very much increased, even if the epidemic be not strong, by a condition of stress among the people. The poor and dependent classes have a higher mortality than among the well-to-do. 'How far this is due to the less deadly effects of malaria or to an increased indirect action of the disease is impossible to say. A feature of the mortality in Amritsar was the relatively large proportion of deaths among adults, especially of the poorer classes. Even the effects of famine are mainly shown through the effects of malaria which follows them'. 'The determining causes of epidemics are excessive rainfall and scarcity, the former is an essential whilst the latter is an almost equally powerful influencing factor'. Christophers (1911a) also showed a suggestive significant correlation between the annual average price of food grains and the annual fever mortality. This correlation has been used by Gill (1928) for a number of years, as one of the factors upon which he bases his annual predictions of the intensity of the autumnal epidemics in the different districts of the Punjab.

Gill (1928) also found that epidemics associated with economic stress are abnormally prolonged, so that the 'fever' mortality, instead of declining to normal in the course of 2 months, remains high for about 5 months. The intensity of an epidemic is enhanced in those areas in which famine or economic stress had prevailed during the preceding two or three years. 'The lowered vitality resulting from famine, if carried to extremes, will lead to death by itself, and hence it is not difficult to understand that the mortality occasioned by a concomitant epidemic is relatively high, apart from any "exaltation" of virulence of the specific parasite, or any diminution in specific immunity'.

White (1909) also points out, in relation to the 1908 epidemic in the United Provinces, that 'no doubt the poorly-nourished people, those whose stamina had been affected by the high prices prevailing for the past two years, fell easy victims to the disease, whereas the rich recovered'. High prices affect the stamina of the people and render recovery slower and relapses frequent. 'The poor suffer most as they cannot work and have nothing to fall back on while ill; and the disease was so general that their friends could not help them'. 'The very young and the very old suffered severely'.

Summary.—Conditions of economic stress have been found to have a marked influence in causing a greater incidence and severity of malaria in localities where this disease is present. One of the effects of economic stress is to cause an increased mortality, both direct and indirect, from this disease.

(4) CONCLUSIONS.

(1) While the evidence available is admittedly imperfect, due mainly to the unreliable nature of the vital statistics in India, yet there appears to be little doubt that, under ordinary circumstances, *at least* one million persons die each year from the direct effects of malaria.

(2) During years when fulminant or regional epidemics occur, the malarial mortality may be increased by another quarter or half a million deaths.

(3) Sex appears to have little influence upon the malarial death rate in India, apart from the occurrence of untreated, or insufficiently treated, cases among pregnant women.

(4) The mortality from malaria is most marked among infants and children, and is also high at the other extreme of life.

(5) Economic stress has an important influence in enhancing the death rate in malaria.

(b) THE INDIRECT EFFECTS OF MALARIA UPON THE DEATH RATE.

'This (the death rate from "fevers") does not adequately represent the prevalence of the disease, however, for malaria is not essentially a fatal illness, but may be looked on rather as one which in the great majority of cases tends towards recovery, and it is only after repeated attacks that it causes that gradually increasing debility on which some other disease is implanted and ends the scene' (Robertson, 1909).

'Sir Patrick Manson declares that malaria causes more deaths, and more predisposition to death by inducing cachectic states predisposing to other affections, than all the other parasites affecting mankind together' (Howard, 1909).

'Malaria is a benign and protracted disease, which is often complicated and terminated by other maladies, such as pneumonia, infantile diarrhoea, dysentery, ankylostomiasis and so on; and it is often, perhaps usually, impossible to say whether death has been due as much to one of the complications as to the original infection. Even with troops and prisoners, the cause of death in such cases is often ascribed to one or other cause on the evidence of the predominant symptoms at the end' (Ross, 1911).

'It can kill by lowering the resistance of the body so that either the individual easily falls a victim to some other disease *in the future*, or by his weakness from malaria, preventing him following his occupation whereby he himself through insufficient food dies from a future attack and also his dependents from lack of food fall to malaria or some other disease' (Newell, 1913).

'The recorded mortality of a disease frequently does not indicate its true influence on the death rate. This is eminently true of malaria. From its effects, physical and economic, in lowering the general vitality of a community, it is a causal factor in many a death in which it is not the *terminal* factor, the one recorded as the "cause of death". *Mortality statistics do not, then, give the proper weight to this disease as a cause of death*' (Carter, 1919).

'When malaria is present it lowers the vitality of the victims and allows germs of other diseases to attack, sicken and kill them' (Watson, 1933).

The indirect mortality from malaria may be caused either by the infection predisposing to death from other causes in the patients, or may act indirectly on the uninfected dependents of the malarious patient. The latter point has, to some extent, been considered when economic stress was discussed previously.

Wise (1920) likens the victim of malaria to a reed shaken in the wind to whom the slightest change becomes unfavourable weather, his powers of resistance being diminished and his physical stamina undermined.

Numerous workers have pointed out that an increase in the incidence of malaria is followed by a rise in general sickness and in the mortality from other causes. On many occasions it has been observed that as soon as the beneficial effects of anti-malarial measures are felt, there is a distinct, and often conspicuous, fall in the general mortality. Watson (1924) states that, under ordinary circumstances, the death rate in a malarious area is two to five times that of a similar community free from malaria. This worker (Watson, 1933) reports that, after malaria control was started at Klang and Port Swettenham, there was a remarkable diminution not only in the number of deaths from malaria, but in deaths from other diseases, particularly from diarrhoea, dysentery, pneumonia, abscess, kidney disease, anaemia, convulsions in infants, etc.

The report of the Royal Commission on Agriculture in India (1928) points out the importance of malaria as a predisposing cause of death from other diseases in this country. It is stated by Ormsby-Moore (1929) that malaria is the main indirect cause of debility, suffering and death from other causes in the Straits Settlements and the Federated Malay States.

The death rate in infected children after the first year of life is mainly due directly to the intensity of the parasitic infection, although secondary affections may play a rôle in terminating the scene. Among adults, on the other hand, indirect mortality is usually a more common cause of death among individuals weakened by this disease. Kenrick (1911) found this to be the case during an investigation into 300 deaths in an area in the Central Provinces where malaria was hyperendemic.

It must be remembered that malaria may complicate, or be complicated by, any disease known to medical science, and in India, where malaria is so prevalent, such complications are very common occurrences. The combination of two affections makes death more probable.

The rise in the death rate from respiratory diseases in a malarious population has been noted by many observers. Christophers (1912) in the Andaman Islands, as the result of post-mortem examinations, found a close relationship between malaria and fatal lobar pneumonia. Before and during the War, the severity, course and incidence of this type of pneumonia was well recognised on the N. W. Frontier of India, among troops who had suffered severely from malaria in the previous autumn. Hendley (1918) and Newell (1913) report that, in the Punjab, it is difficult to give any idea of the malign influence of malaria upon the deaths from respiratory diseases among the children of that province. Phillips (1925) thinks the high mortality in the U. P. Terai during the winter months is due to the action of pneumonia and bronchitis upon a malaria-weakened population. Stewart and Proctor (1907) found in Bengal that 'persons much debilitated by malaria appear to be predisposed to such diseases as phthisis, dysentery and diarrhoea, so that probably many deaths ascribed to these diseases are primarily due to malaria'.

If one study the detailed vital statistics collected by Brahmachari (1923) from three adjacent malarious localities in a rural district of Bengal, some interesting points emerge. When severe malaria invaded one of these areas (Bokhara), apart from a rise in malarial mortality of over 400 per cent, the

recorded non-malarial death rate rose about $2\frac{1}{2}$ times. In succeeding years when the malarial mortality had fallen again markedly, while the non-malarial death rate also fell, the latter still remained higher than in the adjacent areas. These later effects probably represent the action of other diseases in causing mortality among a population weakened by chronic malaria. When the malarial incidence is at its height, it seems probable that many of its victims succumb to other acute intercurrent infections within a comparatively short period. Other individuals contract more chronic infections, or are left in a condition favourable to contracting them, and are carried off one or two years later—the aftermath of the malarial disease. In the same locality the death rate from pneumonia was many times greater than in adjacent but less malarious areas.

Another factor which affects indirectly the mortality for which malaria is primarily responsible, is that when the wage-earners of the household are sick with this disease, or other illnesses secondary to it, their wage-earning capacity is diminished or stopped. This, among the poorer classes whose resources are minimal, leads to a condition of economic stress, and, if such illness be prolonged, may lead to insufficient food. Such a condition among his dependents is aggravated if the male member of the household dies. This condition of starvation retards or prevents recovery of the sick, and makes the healthy more liable to contract other diseases. This result in many cases, as pointed out by Bentley (1925) and other workers, leads to poverty and often more deaths in the family, and eventually may lead to the family being completely wiped out (*vide* p. 252).

As will be discussed under infantile mortality, the death rate among children is markedly influenced indirectly by illness or death, due to malaria, among their mothers. It is only when the child has had the time and the opportunity to acquire an infection that the direct action of the disease occurs. In the early months of life, infants die mostly from this indirect action (premature births, malnutrition, etc.), while later both this cause and an acquired malarial infection combine to raise the death rate, but the former appears to be more potent in the early months. Among adults the direct action is usually less evident, and larger numbers are usually killed indirectly as the result of intercurrent diseases predisposed to by previous malarial infection. There may be, however, as found by Christophers (1911a) in the epidemic at Amritsar, a relatively high death rate among adults, especially those of the poorer classes. As a result of this epidemic in the United Provinces, White (1909) says that 'the general health of a large proportion of the population of these provinces has been seriously and permanently affected, as it cannot be questioned that anæmia, enlarged spleens and malarial cachexia have greatly impaired the constitutions of the attacked'.

INDIRECT EFFECTS OF MALARIA ON INFANTILE MORTALITY.

In British India the infantile mortality is said to be twice as great in districts where there is severe endemic malaria as in relatively healthy districts. Robertson (1912), during his investigations into the causes of infantile mortality in the United Provinces, placed malaria first in importance, 'operating both directly upon the infant and indirectly through the mother'. It is stated by Hehir (1927) that 'it is, directly and indirectly, one of the chief causes of high infantile mortality' in India.

The Public Health Commissioner with the Government of India, in his Annual Report for 1931, has tabulated the annual rates of infantile mortality in 26 different countries for that year. British India heads the list with a rate of 179 per mille of living births. This is 2·7 times as great as that reported from England and Wales. In this year there occurred in British India 1,633,476 recorded infantile deaths, or 24·7 per cent of the total mortality. Of these deaths 29 per cent occurred during the first week of life.

The three main causes of infantile mortality are (a) congenital and developmental defects, (b) alimentary disturbances and (c) infective diseases. Of these the first accounts for nearly all the still-births and deaths under 7 days old, while prematurity plays an important rôle in the first two. The last two factors come into action mainly among older children. In India during the quinquennium 1927—1931, 43·2 per cent of deaths among infants were recorded as due to 'infantile debility and malformations, including premature birth'. The marked action of malaria in causing premature and still-births has already been discussed (*vide* pp. 234-235).

As mentioned previously, malaria may not be a very important factor as a direct cause of deaths during the early months of life. The deaths indirectly caused by this disease during the first year of life, are markedly influenced by (a) the initially lowered vitality of the child, whereby there is an increased liability or susceptibility to intercurrent affections, or (b) diminished power of a sickly mother to suckle her children, or (c) lack of proper attention to the child because of the illness or death of the mother.

Thomson (1931) states 'it is not uncommon in India, for patients with a history of malaria to develop a temperature after child-birth, and in this connection the writer was informed, for example, that in the hospital attached to the Women's Christian Medical College, Ludhiana, Punjab (in the main epidemic area), practically every patient during the fever season had a bout of malaria (usually mild) after labour'. 'Such latent malaria can hardly be without its effect upon the unborn child, even although the latter may be carried to term'.

It has been recognised in most malarious countries that children born of malarious mothers are not so sturdy as those born of healthy ones. This is even apart from any question of premature birth.

Le Dantec (1924) states that, in Algeria, Paulin-Dupuy found that the newly born children of malarious mothers weighed 357 grms. less than those of healthy ones. Pieter in San Domingo reported that 52 children of mothers suffering from chronic malaria without cachexia, weighed 290 to 359 grms. less than normal, and that the mortality among such children was 10 per cent. Of 35 children of mothers with chronic malaria and slight cachexia, the weight was 300 to 400 grms. less than normal and the infantile mortality was 20 per cent, while if the mothers had marked cachexia the mortality was 75 per cent. Abelin in France found that the children of malarious mothers weighed 280 grms. below normal. Laffont and Jahier (1930) record that the full-term children of malarious mothers weigh on an average one-fifth less than normal.

From these figures and the reports of other workers, it is evident that the children of mothers infected with malaria are much less robust than those of healthy mothers. As recorded by Pieter a higher mortality occurs among such children. Blacklock and Gordon (1925) found in West Africa that among 51 normal births in which the placenta showed malarial infection, 25·5 per cent of the children died inside 7 days, while among 93 normal births of uninfected mothers only 5·4 per cent died.

Orgeas (1883) reports that of 317 normally born children of European parentage born in French Guiana from 1859 to 1882, 40 left the colony, while 238 died, mostly in infancy. The children still alive at the end of that time, were without exception weakly and poorly developed.

The infantile mortality in areas where malaria is endemic has been studied by various workers in India.

The causes of infantile mortality in the Terai of the United Provinces were investigated by Robertson (1909). He concluded that 'the group of infantile diseases—inanition, premature birth and infantile convulsions—is at present of special interest, because the two former are representative of the effects of malaria almost wholly and the latter also though in a lesser degree. Inanition includes all those cases where the mother either died or has a poor supply of milk for the child, and the latter as a consequence was practically allowed to starve to death'. 'In inquiries into the deaths of children an attempt was always made to get an interview with the mother, and several very definite histories of premature birth were obtained, and with two exceptions the mother had been having attacks of malaria. The deaths under this head therefore are practically all directly due to malaria'. The group 'inanition, 'premature birth and convulsions' was responsible for 8.6 per cent of the total deaths recorded in this area, of which 'convulsions' accounted for 1.8 per cent.

The same worker (Robertson, 1910), during an investigation at Nagina, found that the mortality reported from 'premature deaths, born weakly and inanition' equalled 12 per cent of the total mortality from all causes, and concluded that malaria was responsible for practically all these deaths. He also considered that a large proportion of the deaths reported from 'dysentery and diarrhoea' were indirectly due to malaria, especially in the later months of the year.

• In an enquiry conducted by Robertson (1912a), in conjunction with Graham, into the death rates in four different towns in the United Provinces, 661 deaths in infants under the age of one year were investigated. Of these deaths 149, or 22.5 per cent, were considered to be due to malaria directly, and there were in addition another 214 in which the cause of death was reported as 'born weakly', etc. His conclusions were that the majority of the latter were indirectly due to malaria, *i.e.*, a total of nearly 56 per cent of the infantile mortality due to this disease, directly or indirectly. He, therefore, classified malaria, operating both directly on the infant and indirectly on the mother, as the chief cause of infantile mortality in these towns.

In another paper, this worker (Robertson, 1912b) pointed out that, as births increase, deaths among infants also increase in a constant ratio. The chief exception he found to this was during the malaria season, when the ratio of deaths among infants rose somewhat. This, he considered, was due more to the indirect effects of malaria than to its direct action.

Graham (1910a, 1910b), in enquiries at Kosi and Kairana, also concluded that deaths recorded as due to 'born weakly, born prematurely and inanition', were closely related to the prevalence of malaria. From his later work at Meerut (Graham, 1913), he thinks that one can legitimately add half the deaths reported under these headings to that of the malarial mortality.

Phillips (1925) comments upon the high infantile mortality in the hyper-endemic tracts of the U. P. Terai. He found this very high both among the indigenous population (401·9 per mille) and among the immigrants (533·7 per mille). In the former people during the period 1919—1923, it averaged 16·8 per mille of the population, and among the latter 26·8 per mille. He thinks this difference is probably accounted for by the lower susceptibility of the indigenous population to malaria. He also considers that in both instances malaria is probably responsible for this mortality, and concludes that if this disease is not directly the cause of the mortality, it, at least, plays a prominent part by lowering the general vitality of the people.

Kenrick (1911) points out that in the hyperendemic areas of the Central Provinces, there is a proportionately greater mortality among infants of the 2-3 year age group. The deaths of children in endemic areas constitute 55 to 65 per cent of the total mortality from all causes. Horne (1913) also noted that in areas of the Madras Presidency where malaria was known to be endemic, there is a high infantile mortality. Daniels and Wilkinson (1909) state that 'among natives the infantile mortality is very high in any place where malaria is prevalent'.

Dyson (1911) reports that in the 'wet' talukas of the Bombay Presidency, 75 per cent of the deaths among infants occur under 1 year of age, as compared with 37 to 48 per cent in the 'dry' areas. He also states (Dyson, 1912) that in Bombay City 34·6 per cent of the infantile mortality occurs in the first 30 days after birth, while in Kanara, a malarious tract, it is as high as 75 per cent. In the same city Nerurkar (1930) found that 'infantile debility and malformations, including premature births', were responsible for 38·4 per cent of the infantile mortality, while respiratory diseases were recorded as causing 34·9 per cent of deaths. The former causes were the main feature of deaths during the first month of life, and the latter in the next eleven months. He thinks this high mortality must be due to some other factor which affects all classes, apart from poverty and ignorance. This factor he believes to be malaria.

Brahmachari (1923) states that in the villages studied by him in Bengal, the infantile mortality seemed to be little affected by deaths from diseases other than malaria. In the Bokhara circle, the infantile mortality was 191 per mille of births before the outbreak of malaria, but rose to over 700 when this disease was at the height of its incidence. During the same year, in an adjacent circle where the incidence of malaria was on the downgrade, it was only about 180. In the localities studied by him, 37·2 per cent of the total infant deaths occurred during the first week of life, and half the total deaths were due to 'debility at birth'. He concluded that the infantile mortality 'was markedly correlated to malarial mortality, varying with it directly'.

Barrowman (1934) reports that in the Federated Malay States, as the result of a 30-year campaign against malaria in the estates, the infantile mortality has fallen from 900 to 130 per mille, which gives some indication of the effects of malaria in causing deaths among infants.

Apart from the increased liability of a physically weak child, born of a malarious mother, to contract intercurrent affections and die, there is also the indirectly injurious effect produced on the child by such a mother. In the Report of the Royal Commission on Agriculture in India, it is stated that

'there can be no doubt, too, that malaria, in lowering the vitality of the mothers, is one of the principal causes of the high infantile mortality from which Bengal suffers'.

The suppression of lactation, as a cause of infantile death, is recognised by the lay public in many parts of India. The debility of the mother caused by repeated attacks of malaria has a marked effect in diminishing or suppressing the milk. Even if the child be born alive, and comparatively healthy, it is liable to die of shortage of milk and inanition.

Many malarious mothers may die, or be so incapacitated by the combined effects of malaria and parturition that they are unable to give their infants proper attention. This unavoidable neglect must be responsible for a large number of deaths among infants in malarious areas. This effect is graphically described in the Report of the Burdwan Fever Commission of 1879 (*vide* Fry, 1912). It leads to inanition, debility, alimentary disturbances, respiratory troubles and other intercurrent affections, which prove fatal in very many instances.

SUMMARY.

There is very much evidence to support the belief that malaria has a very marked indirect effect in increasing the general mortality from other diseases in the localities where it is prevalent.

This effect is especially marked among children, and malaria appears to have a very definite influence in raising the infantile mortality rate.

(c) THE INFLUENCE OF MALARIA UPON THE TOTAL MORTALITY.

Can any estimate be made of the number of deaths which are caused, both directly and indirectly, by the action of malaria?

In the malarious portions of the Bengal Duars, Rice (1931) estimates that on tea gardens 55 per cent of the recorded deaths in hospital are directly due, or secondary to, this disease.

Stokes (1913) says that endemic malaria in the Central Provinces increases the total death rate by 10 per mille of the population. Kenrick (1914) studied carefully the vital statistics of 34 'healthy villages' (spleen rate 4 per cent) in these provinces, and compared them with those of 33 'hyperendemic' villages (spleen rate 80 per cent). He found that the average non-epidemic death rate in the former villages was 25 to 30 per mille, as compared with 38 to 45 in the latter, *i.e.*, an increase of about 10 to 15 per mille.

Clyde (1931) has compared the death rates in certain parts of the United Provinces for the quinquennium 1924—1928, and has found a relationship between the death rate and the intensity of malaria, as judged by the spleen rate (*vide* Table V). Similar observations are reported from Java and Malaya.

Clyde's figures show that, as compared with a relatively non-malarious part of these provinces the death rate is raised by nearly 10 per mille of the population, in areas of moderate to high malarial endemicity. In hyperendemic tracts, the rise is over 20 per mille, *i.e.*, the death rate is about double. If we assume that even such a small proportion as an average of 2 per mille of the death rate is caused by malaria, directly and indirectly, in the comparatively

healthy parts of these provinces it seems probable that this disease is responsible by its action for an average death rate of 8 per mille as a minimum.

TABLE V.

The relationship between spleen rates and death rates in the United Provinces.

Locality.	Spleen rate.	Average death rate per mille.*
(i) The relatively healthy plains of Oudh.	Variable but not over 10 per cent.	22
(ii) Less healthy zone in plains of Rohilkhand.	10 to 50 per cent.	31
(iii) Unhealthiest zone of notoriously malarious 'Terai' of U. P.	Average 75 per cent.	36 to 52 (average 44)†

* The average annual death rate for the provinces during the same period was 25·31 per mille (Clyde, 1931).

† Phillips (1925) records a death rate of 39·8 per mille among the Bhuksas (the aboriginal population), and 60 per mille among the Desis (the immigrant population) of the Gadarpur tehsil of this area.

The average annual death rate for all India during the months when malaria is least prevalent is about 21 per mille in most areas, while during the malarious months this may rise to 28 or more. These figures support the view that malaria by its action, direct and indirect, is responsible for an increase in the death rate of at least 8 per mille per annum.

Malaria is pre-eminently a disease of rural areas, whose inhabitants form about 90 per cent of the population of India. The Public Health Commissioner with the Government of India, in his Annual Report for 1931, states that the fever death rate among the rural population was 16·2 per mille as against 7·8 per mille among the urban population. This difference appears to be an almost constant factor in the vital statistics of India. There was no other endemic or epidemic disease reported during 1931 which would appear to account for this great difference in mortality under the heading 'fever'. It, therefore, seems likely that most of this difference (about 8 per mille) may be due chiefly to the fatal effects of malaria, either directly or indirectly.

If one accepts the figure of 8 per mille as a possible estimate of the deaths directly and indirectly caused by malaria, the mortality from this disease in 1931 was 2,172,214 persons.

SUMMARY.

From the evidence available it seems probable that malaria, by its direct and indirect actions, is responsible for at least 2,000,000 deaths each year in India.

(d) CONCLUSIONS AS TO THE EFFECTS OF MALARIA ON MORTALITY.

The evidence available regarding the influence of malaria on the mortality among the population of India has been given and has been discussed in detail. Although this evidence is admittedly imperfect, one appears justified in making the following tentative conclusions from the data at our disposal :—

(1) In ordinary years, malaria is responsible directly for at least 1,000,000 deaths each year, and, in years when severe regional epidemics occur, this figure

may be increased by another quarter to half a million. The fatal effects of the disease fall chiefly on children and infants. The local distribution of the mortality may be markedly increased by conditions of economic stress.

(2) Apart from the direct mortality due to malaria, it has also a marked indirect action by lowering the general vitality of its victims, whereby many of them become more liable to contract other diseases, from the effects of which many of them die at a later date.

(3) There seems little doubt but that malaria, by its combined direct and indirect actions, is responsible for at least 2,000,000 deaths each year in India.

(III) CONCLUSIONS AS TO THE EFFECTS OF MALARIA UPON THE NATURAL INCREASE OF THE POPULATION.

The data which have been collected have shown that malaria exerts a powerful influence in retarding the natural increase of the population in India.

The action of the disease in this respect is shown by :—

(1) its effect in lowering the birth rate by (a) the prevention of conception and by (b) the causation of abortions and premature interruptions of pregnancy; and

(2) its effects in raising the death rate by its direct and indirect actions.

It might be argued that this curtailment of the natural increase of the population should be considered as beneficial in a country like India, which many persons consider to be over-populated already. Even if one admit this debatable point, the aim of any limitation of population is the production of fewer but healthier individuals. If malaria only killed off the unfit persons, one might possibly consider it as 'a healthy pruning' of the people. Unfortunately this is not so. The disease often kills strong and healthy individuals, and even when it does not do so, its action, as will be discussed later, tends to swell the number of 'unfits' in the population.

(To be continued.)

SCHEMATIC TABLE FOR THE IDENTIFICATION OF THE INDIAN ANOPHELINE MOSQUITOES.

Part I.

ADULTS.

BY

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SYNOPTIC table for the identification of the Indian Anopheline mosquitoes was published originally in the form of a wall chart in 1912 by the Central Malaria Bureau, Kasauli (now incorporated into the Malaria Survey of India). As the study of the systematics of the Indian Anophelines advanced and the number of the different species found in India increased, a dichotomous synoptic table in the form of a chart, small enough to be handled easily, was considered impracticable. Consequently, the table was published in the form of a small booklet (in 1916), which was subsequently revised and is now issued as a profusely illustrated Health Bulletin (No. 10, 1931). This synoptic table is very comprehensive, giving supplementary and alternative characters, wherever necessary, for the different species.

Most probably owing to the slight inconvenience of turning over pages of the small booklet, while identifying a specimen, various attempts have been made to evolve a workable chart for the identification of the Indian Anophelines. All these attempts, made mostly by the students who attend the Annual Malaria Class conducted by the Malaria Survey of India, have so far been unsuccessful, as the charts were found to be too unwieldy and cumbersome.

Treillard (1934) has recently published a synoptic table for the identification of the 21 species of Anopheline mosquitoes of Indo-China. By giving schematic representations of the different important diagnostic characters, he has been able to make the table for the Indo-Chinese Anophelines so concise as to cover only a postcard. With the help of his table, identification is no doubt very rapid and fairly easy and it is quite possible to identify most of

the Indo-Chinese species without using a lens. The number of species of Anopheline mosquitoes occurring in India is, however, more than double that found in Indo-China and a number of them are so closely allied that the use of a lens will be essential for the correct identification of most of the species, even when using the chart now published.

Mainly following the same lines as used by Treillard, I have made out a schematic table for the identification of the Indian Anopheline mosquitoes. The main reason for the making of this schematic chart is not as professed by Treillard for his table, *i.e.*, the uncertainty of identification while using a dichotomous synoptic table, but it is put forward as a supplement to the dichotomous table. The synoptic table for the identification of the Indian Anopheline mosquitoes as published in the Health Bulletin No. 10 (Christophers *et al.*, 1931) has been tried for a number of years at the Annual Malaria Class conducted by the Malaria Survey of India, and has been found very easy to use even by a beginner. In the vast majority of cases, if the table be used with care, no trouble should be experienced in identifying any of the Indian species. This new schematic chart is mainly intended for the use of persons working in the field, as it is much more convenient to handle than the one in the form of a booklet. The other advantage of the chart lies in the fact that it gives the most important differential characters for the various species in such a way that one can easily follow them at a glance. Moreover, as the identification of most of the species is dependent on a combination of a number of characters rather than on any one particular characteristic, comparison between the different species becomes very easy. The chart will be useful also for those who receive by post specimens for identification from other arcars, as these may sometimes be damaged in transit. In such cases, as the *important* characters for each species are tabulated in the chart, the absence of any particular structure in any specimen need not prevent the identification of such specimens under most circumstances. As all the main differential characters used in the synoptic table given in Health Bulletin No. 10 have been included in this chart, it enables the worker to use the chart in conjunction with the Bulletin.

The chief differential characters used are :






1. WING (column 1). *Number of dark areas involving BOTH the costa and the longitudinal vein 1.* This character divides the Indian Anophelines into two subgenera (which are shown in column 2). (a) Subgenus *Anopheles*,—those mosquitoes which either have no pale spots on the wing or have less than four dark spots involving *both* the costa and the longitudinal vein 1; (b) Subgenus *Myzomyia*,—those in which the number of such dark spots is always 4 or more.

It is essential that the dark spots should involve *both* the costa and the longitudinal vein 1, as otherwise a difficulty will be encountered in identifying specimens of *A. gigas*, in which species there are usually five dark spots on the wing costa but only three of these embrace *both* the costa and the longitudinal vein 1. To illustrate this character the figure of *A. gigas* wing is given in this column, and should be compared with that of other species belonging to the subgenus *Myzomyia*.

2. HIND TARSI (column 3). *Number or portion of tarsal segments continuously white* (only the four distal tarsal segments have been shown in the chart). So far as the known Indian species belonging to the subgenus *Anopheles* are concerned, the tips of the hind tarsal segments are never white in any of them.

CHART (plate II).

SCHEMATIC TABLE FOR THE IDENTIFICATION OF THE INDIAN ANOPHELINE MOSQUITOES. I. ADULTS.

 . Speckling,  . Mottling,  . Banding,  . Apical and basal banding,  . (NOTE. Distal ends of legs and palps are directed ←)

3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864	865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960	961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998	999	1000	1001	1002	1003	1004	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016	1017	1018	1019	1020	1021	1022	1023	1024	1025	1026	1027	1028	1029	1030	1031	1032	1033	1034	1035	1036	1037	1038	1039	1040	1041	1042	1043	1044	1045	1046	1047	1048	1049	1050	1051	1052	1053	1054	1055	1056	1057	1058	1059	1060	1061	1062	1063	1064	1065	1066	1067	1068	1069	1070	1071	1072	1073	1074	1075	1076	1077	1078	1079	1080	1081	1082	1083	1084	1085	1086	1087	1088	1089	1090	1091	1092	1093	1094	1095	1096	1097	1098	1099	1100	1101	1102	1103	1104	1105	1106	1107	1108	1109	1110	1111	1112	1113	1114	1115	1116	1117	1118	1119	1120	1121	1122	1123	1124	1125	
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The species belonging to the subgenus *Myzomyia*, however, resolve into four groups on this character. (a) With tip of hind tarsi not white; (b) with tip of hind tarsi white, having only one segment or less continuously white; (c) having two terminal segments continuously white; (d) having three terminal segments continuously white.

3. FORE TARSI (column 4). *With broad conspicuous pale bands or with very narrow inconspicuous or no bands on them.* (Only the four proximal segments have been shown in the chart.) This character distinguishes four species, viz., *subpictus*, *vagus*, *sundaicus* and *stephensi* from all the other Indian *Myzomyia* having the tips of hind tarsi black. These are further differentiated from one another on the speckling of the tibiae and the femora, and on the banding of the palps of the female. All species, having the tip of hind tarsi white, show the banding of the fore tarsi, though the banding in most of them is not as conspicuous as in the four species mentioned above.

4. TIBIAE AND FEMORA OF HIND LEGS (column 5). *All black or with marked pale spots of the nature of speckling or mottling or with pale bands.* The bands may be basal, apical or median. Speckling has been indicated in the chart by small white dots, while mottling, as found in *pulcherrimus*, has been shown by large irregular spots. A marked 'knee-spot' at the tibio-femoral joint, wherever present, has been shown as a white band*.

5. PALPS OF FEMALE (column 6). *Number, relative width and the distribution of pale bands.* The relative widths of the various pale bands are not drawn to scale in the chart. In the diagrammatic representation of the pale bands in the chart, the width given only serves to differentiate between a narrow and a broad apical pale band. The width of the apical pale band, as shown for *A. superpictus*, is meant to indicate a 'narrow apical band', while any band wider than this denotes a 'broad band'.

A combination of the above five characters is quite enough for the identification of most of the Indian species, but in some these five structures have a combination of similar characteristics common to more than one species. Additional characters have, therefore, been given in column 7 under 'Other Diagnostic Characters' for all those species which one is unable to identify with certainty on the five chief characters dealt with already. This column is divided into two parts, in the first the names of the structures are given, while in the second the important diagnostic feature of that structure for each species is described. Those species, belonging to the subgenus *Myzomyia* having the hind tarsi black and fore tarsi without broad pale bands, have been divided into two groups: (a) Species in which the thorax is covered with obvious scales; and (b) those in which the thorax bears hairs or hair-like scales only. The species belonging to the latter group, and in which the tip of the palp is pale, are subdivided into two sections. In three of these species the longitudinal wing vein 3 is black, while in four this vein is extensively pale.

Besides these characters, special features of the wing are also given (in column 8) wherever considered necessary. This column has also been divided into two parts. In the first, the name of the important feature of the wing is

*The broad white band shown in this position in the case of *A. annandalei* indicates a tuft of large white scales which at first sight might appear to be a white band in the actual specimen.

given, while in the second, either the special characteristic of the part is explained, or a figure of the wing has been given for clarity.

A number of species may vary in the degree of paleness on certain structures (*viz.* palps and legs). Some of the most important of these variations are given below.

A. superpictus : An extra dark band may appear in the region of the apical pale band on the palps of the female, so that the palp either bears four pale bands or appears similar to that of *A. multicolor*. In the former case, the specimen can be identified on the combination of the characters already dealt with, while in the latter case the scaling on the thoracic fossa and the dark spots on the longitudinal vein 6 are enough to differentiate the two species.

A. leucosphyrus and *A. tessellatus* : The pale tips of the hind tarsi of these two species are sometimes not very obvious. In such cases the presence of speckling on the legs, four-banded palps in the female, together with the characteristic spotting of the longitudinal veins 5 and 6, are enough to differentiate these two species correctly.

A. annularis : Specimens of this species showing only two hind tarsal segments continuously white are often met with particularly during the winter months in the Punjab (var. *adieii*). In such cases the unspeckled legs, banding of the palps and the characteristic pattern of the wing are by themselves enough for the identification of this species.

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SCHEMATIC TABLE FOR THE IDENTIFICATION OF THE INDIAN ANOPHELINE MOSQUITOES.

Part II.

FULL-GROWN LARVÆ.

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[3rd July, 1935.]

THE identification of most of the adults of the Indian Anopheline mosquitoes is dependent upon ornamentation which can be easily studied under a lens. In some cases the main differential characters are so gross that it is possible to identify a specimen even with the naked eye. In the case of larvæ, on the other hand, the diagnostic characters, *e.g.*, the form of certain fine hairs which arise from different parts of the body of a larva, are so small that, in order to identify a larva correctly, each has to be examined under a microscope, often under a fairly high magnification ($\times 300$). For this reason the identification of the larvæ of Indian Anopheline mosquitoes is comparatively much more difficult than that of the adults, and a number of workers prefer to breed them out so as to identify them as adults rather than as larvæ. On account of the high mortality, which may sometimes occur during this process, much valuable information may however be lost.

Though the adults have been the subject of much detailed systematic study for a long time, the knowledge of larvæ was, until comparatively recently, very incomplete. The necessity for a complete diagnostic table for the identification of all the Indian species had been felt by field workers for many years.

As the result of an exhaustive systematic study of the larvæ of all the known Indian Anopheline mosquitoes, two identification tables were published by me in the form of a Bulletin (Puri, 1930). A third synoptic table, which to some extent showed the mutual relationship between the various species, was

also compiled to supplement the above two tables (Puri, 1931)*. The two former tables have since been revised in the second edition of the Health Bulletin No. 16. These tables have been used for a number of years at the Annual Malaria Class conducted by the Malaria Survey of India and have been found quite successful and fairly easy to work.

The present schematic chart for the identification of the larvæ of the Indian Anophelines is compiled as a supplement to the dichotomous tables already published. It is intended as a means of ready reference for persons working in the field. The most important differential characters, along with some supplementary ones for the various species, are tabulated in the chart in such a way that one can follow them easily at a glance. With the help of this chart comparison between the different species becomes very simple.

The various hairs, the forms of which usually serve as very important diagnostic character, often fall off. This is most commonly the case in specimens which have been preserved for some time. It may also happen when a large number of living larvæ are kept in a small space, under which circumstances various important hairs may be nibbled off by other larvæ. The identification of such denuded larvæ, at times, may become very difficult when using the ordinary synoptic tables. As in the case of the adults the identification of any species is based on a combination of a number of characters rather than on any particular one. Consequently, while using this chart in which all the important characters are tabulated, the fact that any hair is absent or obscured need not prevent the identification of most of full-grown larvæ. The main differential characters used in this chart are those on which three synoptic tables are based, so it can be used in conjunction with these tables.

DISCUSSION OF CHARACTERS USED AND DEPICTED IN THE CHART.

In this chart eight main diagnostic characters have been used, and where necessary these have been supplemented by other points of value in the confirmatory identification of certain species.

1. RELATIONSHIP OF THE BASES OF THE INNER CLYPEAL HAIRS (Column 1).

The larvæ of the Indian Anophelines can be divided on this character into two subgenera (shown in column 2).

(a) SUBGENUS *Anopheles*—*Those species in which the bases of the inner clypeal hairs are close together* (Fig. 1). If they are not touching each other, the distance between them is never more than that between the bases of the inner and the outer clypeal hairs of one side (Fig. 2).

(b) SUBGENUS *Myzomyia*—*Those in which the bases are well separated, i.e., the distance between them is always more than that between the bases of the inner and the outer clypeal hairs of one side* (Fig. 3).

*The reader is referred to this work for a detailed and profusely illustrated description of the known larvæ of all the Indian species of Anopheline mosquitoes.

SCHEMATIC TABLE FOR THE IDENTIFICATION OF THE INDIAN ANOPHELINE MOSQUITOES. II. FULL GROWN LARVAE.

[illegible]

2. ANTENNAL HAIR (Column 3).

Its form and position on the shaft of the antenna.

In most of the species belonging to the subgenus *Anopheles* the antennal hair may be branched, in which case it *always* arises from the *inner* surface of the antenna. In the first six species and varieties, mentioned in the chart, this hair is fairly short and branched from its base, and it arises from the dorso-internal surface of the antenna very near its proximal end (Fig. 7a). In the next four species, however, it is fairly long with long pinnately arranged branches and arises from the internal surface at about the middle of the antenna (Fig. 7b). In all other species of the Indian Anophelines it is minute and simple, and *always* arises from the dorso-external surface of the antenna (Fig. 7c).

3. FORM OF FRONTAL HAIRS (Column 4).

This character is important mainly for the identification of those species which breed in tree-holes. In these species such hairs are very short and may be simple or branched. Only in one of them, *viz. annandalei*, is one of the pairs long—it is also simple. In all the other species, not breeding in tree-holes, all the three pairs of frontal hairs are long and pinnate. The only known exception is *A. turkhudi*, in which these hairs bear very few branches (2—8).

4. RELATIVE SIZE OF TERGAL PLATES* (Column 5).

On this character five species, *viz. minimus, fluviatilis, varuna, aconitus* and *jeyporiensis*, are distinguishable from all other Indian species belonging to the subgenus *Myzomyia* as well as *Anopheles*. In these five species the anterior tergal plates are exceptionally large, as compared to those of any other species. In four of them the posterior border is convex and the plates include the 'small median chitinous spot' in them, while in the fifth (*jeyporiensis*) the posterior border is concave, with the median chitinous plate lying behind it. In all the other species the anterior tergal plates are very small.

5. FORM OF CLYPEAL HAIRS (Column 6).

This column is divided into three sections, one for each of the three hairs—inner, outer and posterior clypeal hairs. In each of the three columns either (a) a diagram showing the form of the hair, or (b) the number of branches (if the hair be branched), is given for each species. Those species belonging to the subgenus *Myzomyia*, in which the anterior tergal plates are fairly small, have been divided into two groups: (A) Those species in which the inner and the outer clypeal hairs are either simple or have fine inconspicuous fraying, and (B) those in which they show conspicuous branches. The species in the latter group have been further subdivided into two sub-groups, on the morphology of the outer clypeal hairs:—(a) those species in which the outer clypeal hairs bear very short branches (being less than one-fourth the length of the hair itself), and (b) those in which the branches are very long, often about half the length of the hair.

* The anterior tergal plates for each species are shown as shaded areas in the chart (column 5), while the median chitinous plate is described as a dark spot. These structures have not been drawn to scale. The posterior 'oval plates', whenever present in a species, have also been depicted as small dark spots.

6. PALMATE HAIRS (Column 7).

This column is further subdivided into eight sections, one for each of the first seven abdominal segments and one for the metathorax. The number of segments with fully developed or poorly developed palmate hairs, or on which palmate hair is not differentiated, is shown for each species. A completely dark area indicates fully developed palmate hair, while a shaded or a blank area means a poorly developed, or not differentiated, palmate hair respectively. If a part of a segment has been left blank on the chart while the rest is shaded, it means that in some cases the palmate hair on that segment is not differentiated.

7. FORM OF LONG THORACIC PLEURAL HAIRS (Column 8).

On each of three thoracic segments there is a group of pleural hairs arising from a tubercle, situated on the ventro-lateral surface on each side. A certain number of hairs in each group are very long, and it is the morphology of the latter hairs only which has been found of any diagnostic importance. In this column either a diagram showing the form of these long hairs has been given, or the form of each of the long hairs has been shown schematically for each species. Although the form of these pleural hairs may only be needed as a supplementary character for some species, it will be found very important in the diagnosis of a number of others.

8. RELATIVE LENGTH OF THE FILAMENT OF PALMATE HAIRS (Column 9).

The relative length of the filament has been given only for those species in which the filament is well differentiated. It will be found useful as a diagnostic character for some species, but has been given only as a supplementary character for the others.

9. OTHER DIAGNOSTIC CHARACTERS (Column 10).

A combination of the above eight characters is enough for the differentiation of a considerable number of the Indian species, but some others show similar combinations of these characteristics in more than one species. Additional characters have therefore been given in column 10—'Other Diagnostic Characters', for all those species which one is unable to identify with certainty on the eight chief characters dealt with already. This column is divided into two parts, in the first the names of the structures are given, while in the second the important diagnostic feature of that structure is described for each species.

IMPORTANT VARIATIONS AND ABNORMALITIES.

Specimens showing extreme variations are not uncommon among larvæ of the Indian Anopheline mosquitoes. Some of the most important of those variations which may cause confusion in identification are given below.

In very rare cases among the larvæ belonging to the subgenus *Myzomyia*, the distance between the bases of the inner clypeal hairs is equal to that between the bases of the inner and the outer clypeal hairs of one side. In such specimens the presence of the minute simple antennal hair arising from the *dorso-external* surface of the antenna *together with* the long feathered frontal hairs, will at once show that the specimen belongs to this subgenus.

In very exceptional specimens belonging to the subgenus *Myzomyia*, the antennal hair may be branched. In such cases its origin from the dorso-external surface of the antenna at once distinguishes it from the similar hair of the subgenus *Anopheles*, in which it is *always internal* in origin when branched.

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THE EFFICACY OF VARIOUS INSECTICIDAL SPRAYS IN THE DESTRUCTION OF ADULT MOSQUITOES.

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INTRODUCTION.

It is now a matter of common knowledge that many insects, such as house-flies, mosquitoes, sandflies, fleas, etc., are important factors in the dissemination of several human diseases. Apart from any question of the spread of disease, these insects may give rise to considerable personal discomfort from their bites or their habits. For these reasons, insecticides of various kinds have been in demand for several decades. Within the last few years, a number of insecticidal spraying solutions have been placed on the market, and some have achieved a great popularity, chiefly because of the increased comfort arising from their use.

James (1926) arrived at the conclusion that infected specimens of *A. maculipennis* in Europe usually passed the whole, or the greater portion, of their lives in the houses where they acquired their malarial infection. This observation gave a great stimulus to campaigns for the destruction of mosquitoes in houses in malarious areas. However, more recent observations have shown that such a habit is not universal among malaria-carrying species of Anophelines, and

it is very probable that the extent to which infected mosquitoes remain in houses may vary considerably with the species, the type of building, and other local environmental conditions. If, however, circumstances be favourable, it seems probable that a number of potentially infective insects may harbour in dwellings, for at least some hours, after obtaining a blood meal there*. Under such conditions, the use of an effective insecticide may be of considerable value, not only in adding to the comfort of the inhabitants, but also as a measure against the spread of malarial infection†.

While numerous mosquitoes may be killed by swatting, this is not an ideal method, for such insects often tend to hide in dark corners and crevices, where they may escape destruction.

For a more wholesale eradication of mosquitoes from buildings, two methods—fumigation and spraying—have been advocated. Workers seem agreed that, for most practical purposes, spraying is preferable to fumigation (*vide* Covell, 1931, 1934).

Many of the insecticidal sprays in common use were originally devised against agricultural pests or against house-flies. Steudel (1911) called attention to the neglect of such measures in malarial prevention. Following upon this, Giemsa (1911, 1912, 1913, 1914) carried out a series of experiments into the efficacy of various kinds of spraying solutions for the destruction of mosquitoes. As a result of his work, he recommended a mixture consisting of tincture of pyrethrum, soft soap and glycerine, diluted with water. After attempts to find a less expensive preparation, he reported that the use of a soap solution containing formaldehyde was effective and cheap.

Nowadays there are on the market many proprietary sprays recommended by the makers for the destruction of mosquitoes. Unfortunately, all these are not equally effective (*vide* Swellengrebel and Nykamp, 1934; Baber, 1934, etc.) and, in most instances, their prices are so high that they can only be utilised by a comparatively small number of people. This question of cost has proved

* That such a circumstance may occur under certain Indian conditions is suggested by some records kindly supplied to one of us (J. A. S.) by Dr. J. de la M. Savage, M.A., M.D., of the Assam Medical Research Society.

Mosquito collections were carried out in certain menial quarters at Shillong, Assam. Of 823 specimens of *A. minimus* captured in these houses during August, September and October, 549 were dissected at intervals varying from 3 to 11 days after capture. Among these, 65 per cent were found infected with oöcysts only, 34 per cent with sporozoites only, and 27 per cent with both these stages of the malaria parasite, *i.e.*, a total of 127 per cent of potentially infective insects. Of seven inhabitants of these quarters whose blood was examined in September, two adults showed malignant tertian rings, and one child crescents.

In another house, 187 specimens of *A. minimus* were collected during September and October, and of these 94 were dissected in from 3 to 10 days after their capture. Among these, 27·7 per cent were found to harbour oöcysts only, 4·2 per cent sporozoites only, and 10·6 per cent both oöcysts and sporozoites, *i.e.*, a total of 42·5 per cent of potentially infective specimens of *A. minimus*!! In this house one child showed crescents in its blood and one adult malignant tertian rings.

It is evident from these observations, that the use of an effective insecticidal spray, or other method for the destruction of adult mosquitoes, should be of great value in hindering the dispersion of potentially infective insects, under such circumstances.

† Such a procedure would probably be very useful in military and other tented camps in malarious areas. Under such conditions, it is not uncommon to find enormous numbers of Anophelines collected inside the tents in the early morning. Systematic destruction of such insects by spraying would probably have a marked effect in hindering the dispersion of potentially infective mosquitoes.

a stumbling block to the more widespread use of those sprays which have been found satisfactory. To overcome this drawback, various workers have carried out investigations in attempts to find some form of spraying solution, which could be easily and cheaply prepared for use in the prevention of malaria.

In most of the sprays that have been recommended as useful by different workers, mineral oils of the nature of kerosene have formed the vehicle for the insecticidal substance used. Apart from aqueous solutions of such substances as cresol, lysol and formaldehyde, oily mixtures of carbon tetrachloride, methyl salicylate, naphthalene and various other aromatic substances have been the chief insecticides recommended for use, either alone or in combination* (*vide* Brug and Van Slooten, 1927; Fraga, 1928; Mansell, 1930; Baber, 1934, etc.). More recent work goes to show that none of these substances can be considered as satisfactory, nor do any of them equal in efficacy the best type of proprietary preparation (Holt and Kintner, 1932; Swellengrebel and Nykamp, 1934; Baber, 1934). Although many of these solutions will knock down and stupefy temporarily those mosquitoes with which they come in contact, a similar effect may be produced by kerosene oil, or even water, under the same conditions. Unfortunately many of such insects recover rapidly and will fly off, unless swept up immediately and burnt.

The combination of extracts of pyrethrum with several of these substances has been found to give an increased efficiency, in that a much smaller number of insects recovered afterwards. Experiments conducted by different workers go to show that the increased and effective toxicity of such mixtures is almost entirely due to the element introduced with the pyrethrum extracts.

It appears from the work quoted by Gnadinger (1933)† that most of the effective household sprays on the market are composed chiefly of extracts of pyrethrum in kerosene oil, with some added perfume. It is also pyrethrum which provides the chief insecticidal principle in many of the locally-prepared sprays which have been recommended in recent years (Holt and Kintner, 1932; Baber, 1934; Swellengrebel and Nykamp, 1934; Michel, 1935).

The evidence available goes to show that probably the most powerful insecticide known is present in the flowers of *Pyrethrum* sp. In recent years commercial firms have been able to produce a concentrated extract of the active principles of such flowers, which is available on the market at comparatively cheap rates.

It is one of such commercial extracts—'Pyroicide 20'—diluted with kerosene oil, which has been used mainly in our experimental work. With this substance we have been able to manufacture locally, at a comparatively cheap rate, an insecticidal spraying solution, which appears to be equal to the best proprietary preparations in the destruction of mosquitoes. Other workers have used different concentrated extracts of pyrethrum, such as 'liquid extract of pyrethrum' and 'Pyagra Concentrate' (Baber, 1934), 'X 10', and 'Pyfume'

* Covell (1931, 1934) has given a summary of the work on the use of spraying solutions for the destruction of mosquitoes, with many references. Gnadinger (1933) has a long list of the various substances which have been tried as fly-sprays. Those interested should consult these articles for further details.

† Gnadinger's monograph is a mine of information on all points connected with the insecticidal properties of pyrethrum. Unfortunately it only came to our notice when our experimental work was finished. However, we have made much use of the information given by him in the preparation of the text of this paper.

(Swellengrebel and Nykamp, 1934), and 'Pyrocide 40' (Michel, 1935), in the preparation of their locally-made solutions, and there is no reason to suppose that many of these would not be as effective as Pyrocide 20 for this purpose.

Before considering the experimental evidence for the value of our insecticidal solution, it is necessary to discuss the insecticidal properties of extracts of pyrethrum, and the methods of preparing spraying solutions from them.

THE INSECTICIDAL PROPERTIES OF PYRETHRUM FLOWERS.*

The use of pyrethrum flowers for insecticidal purposes appears to have originated in Persia. It was first introduced into Europe early in the nineteenth century. At that time and for many years afterwards, a powder prepared from the flowers was used for domestic purposes.

About 1919 in America, kerosene extracts of the flowers, employed as a spraying solution, began to displace the powder for household use. As a result, a large number of proprietary insecticidal sprays have now been placed on the market, in which the chief toxic element is mainly derived from pyrethrum.

'Pyrethrum is particularly adapted for controlling household insects because it is non-poisonous to man, although to insects its active principles are among the most toxic substances known' (Gnadinger, 1933).

After every other attempt had failed, Staudinger and Ruzicka (1924) managed to isolate two active principles from pyrethrum, and solved the problem of their chemical nature. These principles they called 'Pyrethrin I' and 'Pyrethrin II'†. For a more complete account of the chemical aspects of this work, the reader is referred to Gnadinger (1933).

'The pyrethrins are viscous liquids, soluble in petroleum ether, ether, alcohol, acetone, ethylene dichloride and most other organic solvents'. For commercial purposes the solvent which has been found most practicable and effective is probably ethylene dichloride. The oleo-resin extracted with this solvent 'contains practically all the pyrethrins that were present in the flowers'.

Of the several solvents tried by them, Swellengrebel and Nykamp (1934) found petroleum ether the most satisfactory in extracting the active principles from pyrethrum flowers. It is this solvent which is said by Gnadinger (1933) to extract less inert matter than any other. Holt and Kintner (1932) record good results with chloroform as an extractive.

The exact method by which pyrethrum acts in killing insects has not been determined with any degree of certainty. There is, however, much evidence to suggest that it is a nerve poison, and that it may exert its toxic action by direct penetration of the integument, even if not taken in through the respiratory apparatus. For this reason, Gnadinger (1933) says that 'care should be taken to hit as many insects as possible (with the spraying solution) since pyrethrum is primarily a contact spray'. Swellengrebel and Nykamp (1934) also note the effect of the contact of the cloud of minute droplets produced by spraying.

* We are indebted to the monograph of Gnadinger (1933) for much of the information given here.

† These must not be confused with 'pyrethrine', the name given in France to an oleo-resin obtained from pyrethrin, nor with 'pyrethrin', the active principle of pellitory root.

Pyrethrum-oil sprays were tried by Baber (1934) against both house-flies and mosquitoes (Culicines), and he reports that 'mosquitoes appear to be remarkably susceptible to insecticides prepared from good quality extract of pyrethrum', even more so than are house-flies. 'A higher kill resulted with mosquitoes than with flies even with one-thirtieth the quantity of insecticide spray'. Swellengrebel and Nykamp (1934) also found that mosquitoes (*A. maculipennis*) were more susceptible to pyrethrum than house-flies or *Stomoxys calcitrans*. They think, therefore, that mosquitoes are too easily killed to form good experimental insects for testing the toxicity of spraying solutions. It is stated by Brug and Van Slooten (1927) that Anophelines appear to be less resistant to insecticidal sprays than are Culicines.

POINTS TO BE AIMED AT IN THE PREPARATION OF AN IDEAL SPRAYING SOLUTION FOR USE AGAINST ADULT MOSQUITOES.

While many insecticidal spraying solutions, mainly 'fly-sprays', have been placed on the market, or prepared locally, they do not all fulfil the requirements necessary for general use in the destruction of adult mosquitoes.

The main desiderata of an ideal mosquitocidal spray would appear to be the following combination of characters :—

- (1) high toxicity to mosquitoes,
- (2) harmlessness to man,
- (3) low cost, and
- (4) ease of application.

(1) HIGH TOXICITY TO MOSQUITOES.

(i) The spray should, under ordinary conditions of use, cause a high percentage of *deaths* among mosquitoes, and not merely stupefy them and allow them to recover later.

(ii) As many mosquitoes usually hide behind curtains and other draperies, on clothes, under beds, behind furniture, etc., an ordinary insecticidal spray, which may be very good against house-flies and which in many instances depends for its action on 'direct hits' on the insect, is not always suitable. While a room or habitation is being freed of mosquitoes, there would usually be comparatively few chances of obtaining such direct-contact action. It is, therefore, necessary that the insecticidal spray should act powerfully, either by the diffusion of its volatile elements or of fine droplets suspended in the air, so as to reach hidden insects, or should cause such insects to leave their hiding places, so as to come in contact with the more concentrated cloud in the open parts of the room.

(iii) As will be discussed later, the efficacy of some spraying solutions may vary considerably with changes of temperature and humidity. It is desirable, therefore, that the preparation chosen should be able to act effectively under the meteorological conditions most common when mosquitoes are prevalent in the locality where it is being used.

(2) HARMLESSNESS TO MAN.

(i) While the sprayed mixture should have a strong toxic action against mosquitoes, it should have no toxic or unpleasant effect (conjunctival, nasal,

laryngeal or cutaneous irritation, headache, etc.) on human beings, even when used in rooms occupied by them at the time.

(ii) It should produce neither toxic effects nor nauseating taste if it should come in contact with food.

(iii) It should cause no damage to those articles of furniture, clothing, drapery, etc., with which the spray may come in contact during use.

(iv) It should not be so inflammable as to be liable to cause serious accidents under ordinary conditions of use in households.

(v) Its odour should not be such as to cause any unpleasant effects, nor be objectionable to the inmates of the house, at least for any long period.

(3) LOW COST.

Unless the cost of manufacture and distribution is very low, only a favoured few will be able to afford to use it, and it will be beyond the financial reach of the majority of the afflicted population.

(4) EASE OF APPLICATION.

(i) No elaborate or expensive apparatus should be needed for using the preparation in an effective manner. Such apparatus should be capable of use by the inmates of an ordinary household, even if poorly educated.

(ii) It should be capable of working efficiently in large indoor spaces, without any *elaborate* preparation such as sealing up doors and windows, etc.

(iii) It should be rapid in its action, and require a very short time to apply.

PYRETHRUM SPRAYS FOR HOUSEHOLD USES.

Up to the present, the most satisfactory insecticidal sprays for household use appear to be those containing pyrethrum extracts. The proprietary mixtures employed have been confined, almost entirely, to preparations made by the direct extraction of pyrethrum flowers with mineral oil.

About 1929, researches were started in endeavours to obtain, on a commercial scale, a highly concentrated, standardised product of uniform pyrethrin content and toxicity, which could be used in the manufacture of insecticidal sprays. These investigations were successful, and several of these commercial extracts are being used in the local preparation of household sprays in many parts of the world.

(1) THE USES AND ADVANTAGES OF PYRETHRUM SPRAYS.

According to Gnadinger (1933), 'pyrethrum-oil sprays can properly be recommended for killing flies, mosquitoes, roaches, bedbugs, ants, silverfish and nearly all other household pests, including clothes' moths, their larvæ and eggs. It should be remembered that the spray will only kill those insects which it hits.....' 'The user should also be instructed to sweep up and burn flies, mosquitoes, etc., since a few of the insects knocked down by the spray will usually recover'. 'Pyrethrum-oil sprays can be used where food is handled or stored without danger to the consumer'.

(a) TOXIC PRINCIPLES IN PYRETHRUM SPRAYS.

The killing power of such sprays appears to be due almost entirely to their pyrethrin content. While it has been shown (Gnadinger, 1933; Swellengrebel and Nykamp, 1934) that the percentage of flies killed will increase with the amount of pyrethrum extract in the spray, this increased destruction is not proportionate, and, when a certain concentration of the toxic principles is reached, a large increase in the pyrethrin content results in a relatively small increase in the number of kills.

For commercial fly-sprays, it has been found that a pyrethrin content of 100 to 120 mg. per 100 c.c. is satisfactory. This is equivalent to about 1 lb. good quality flowers per gallon of oil.

(b) THE VEHICLE FOR THE TOXIC PRINCIPLES.

In most proprietary preparations and in those made locally, the vehicle is usually some type of mineral oil. Apart from any inherent toxic action, mineral oil forms a cheap vehicle for the extraction or solution of pyrethrins, and, as will be seen later, the insecticidal properties of such solutions appear to keep better than do those of the pyrethrins in pure form.

The mineral oil used is generally one of the kerosene type. Some workers think that such oil has considerable killing power *per se*, but, as the result of our own experiments and the reports of other workers show, it seems proven that these oils have probably little action under the conditions of actual use, unless the insect is actually drenched with them by a direct hit. According to Gnadinger (1933) the relative toxicity of the oil appears to vary rather with the type of sprayer used, and is more or less independent of the physical constants of the oil. This worker has summarised the literature on the subject, and gives a list of references to original investigations.

There are, however, several factors which must be considered in choosing any sample of kerosene for use as a vehicle for insecticidal substances.

(i) *Volatility.*

The oil used should evaporate rapidly without leaving behind any oily residue or causing marks or stains on the furniture, clothes and draperies with which it may come in contact. Some workers recommend the addition of a certain amount of petrol (motor spirit, gasoline) to the oil to increase its volatility, thus Baber (1934) uses 15 per cent of the former liquid. Any such addition should be used with care, on account of the increase in the inflammability which it may cause in the mixture. If the oil be too volatile it may interfere with the production of a good killing cloud from the sprayer.

(ii) *Inflammability.*

Most of these petroleum-oil mixtures are very inflammable if squirted into an open flame, so users should be warned against this. Apart from this fact, the risk of fire from the employment of household insecticides under the ordinary conditions of use is small, if a proper type of kerosene be used as a diluent.

Gnadinger (1933) states that the National Insecticide Manufacturers' Association of the United States of America has adopted a standard for such

kerosene, which lays down that the 'liquid base should not exceed 48.9°C. (120°F.) in flash-point, as determined by the Tagliabue open cup method'. Oil of a lower flash-point should not be used, as it may prove dangerous for ordinary household work.

There are several brands of burning oil on the market in India which fulfil these requirements. In America the mineral oil used by Michel (1935) was a highly refined spirit, not highly inflammable, and of the kerosene type, which is known commercially as 'cleaning fluid or mineral spirits'.

(iii) Odour.

Any odour produced by the oil should dissipate rapidly. In some cases (*vide infra*) certain perfumes have been added to the mixtures, either to mask the odour of the oil, or in attempts to increase their toxic effects. Both Griffiths (1935) and Michel (1935) point out that the smell of insecticides employed in aeroplanes may increase the tendency to air sickness.

(c) ADJUVANTS ADDED TO PYRETHRUM SPRAYS.

Many of the sprays recommended for household use contain a certain amount of a volatile perfume base. This is added in many cases in an attempt either to aid the insecticidal action of the mixture, or to disguise its smell, or for both purposes.

If such sprays are to be used in the neighbourhood of food, perfumes should be avoided. Under most conditions it seems advisable that the substance added should dissipate rapidly and should not linger indefinitely in the room sprayed. In the case of the mosquito nuisance, it is probable that the addition of some substance which has a repellent action against these insects, might be a distinct advantage, especially if its smell be of such a nature that it is not disagreeable to the inhabitants of the building sprayed.

Gnadinger (1933) gives a list of about 150 different odorous substances which have been added to fly-sprays to increase their killing properties. It seems probable that few of these have much action as compared with the toxic effect of the pyrethrins.

Baber (1934) reports that oil of citronella increased the value of pyrethrum sprays against mosquitoes, and it is probable that either this oil, or oil of pine, in strengths of 4 to 5 per cent, would also be useful from the repellent action of these aromatic substances. Swellengrebel and Nykamp (1934) recommend the addition of oil of sassafras in strengths of $\frac{1}{2}$ to 1 per cent, for they believe the odour of this substance makes the insects leave their hiding places, and so they are more readily killed by the spraying solution.

(2) FACTORS INFLUENCING THE TOXICITY OF PYRETHRUM SPRAYS.

Apart from its pyrethrin content there are various factors which may influence the apparent toxicity of pyrethrum sprays. 'It was gradually discovered that the vitality of the flies, the type of sprayer, temperature and humidity as well, of course, as the concentration of the spray used, were all factors affecting the toxicity of the spray' (Gnadinger, 1933).

(a) DETERIORATION OF THE SOLUTION.

Gnadinger (1933) summarises the results of a series of experiments from which he concludes that 'pure pyrethrins decompose rather rapidly on exposure to air and sunlight or ultraviolet light'. 'They are also rapidly decomposed when heated to 100°(C.) in air'. 'When exposed to sunlight in nitrogen they do not decompose appreciably in 12 days'. He found, however, that 'although these compounds are decomposed by heat when isolated in the pure condition, they are apparently not injured by heat when dissolved in ethylene dichloride or mineral oil'. 'Concentrated pyrethrum extract made by this process (the commercial ethylene-dichloride one) retains its strength when properly stored. Such an extract, containing 1,800 mg. of pyrethrin per 100 c.c., was stored indoors in tin cans and iron drums for 13 months at 26°(C.) to 35°(C.)' with no apparent loss of toxicity, when tested by the Peet-Grady method against flies.

Gnadinger (1933) also carried out a number of experiments on the effects of storage upon the toxicity of pyrethrum household sprays. These sprays were prepared by extraction of pyrethrum flowers with kerosene by percolation. He reports that—

(i) portions of this extract were packed in tin cans, and in flint, amber and blue glass bottles. Three packages of this kind were stored out of doors in an eastern aspect for 13 months, during which time the temperature varied from -29°C. to 43°C.,

(ii) three tins were also stored indoors, unexposed to direct sunlight, at a temperature of 26°C. to 35°C.

The toxicity of these samples was then tested against house-flies by the Peet-Grady method. While it was found that those stored in tins and amber bottles showed no loss of toxicity, flint and blue glass bottles were found unsatisfactory for storage.

This worker also reports that, when certain highly refined oils are used as solvents for pyrethrum extracts, a very light precipitate of inert resin may form, which can be removed by filtration.

(b) THE DIFFUSION OF THE CLOUD OF SPRAY.

The actual action of the spraying solution probably depends, for the most part, upon effects following direct contact, with the insect, of minute particles in the cloud produced by the atomizer or sprayer. It is obvious, therefore, that the nature of the cloud will have a considerable influence upon its insecticidal efficacy.

As is pointed out in the discussion on methods of testing insecticidal solutions, the type of sprayer may have a distinct effect upon the uniformity of the results obtained in such tests. Gnadinger (1933) considers that the relative toxicity of spraying mixtures may vary with the type of sprayer employed, and that a sprayer which is suitable for one kind of spray may not be so good for another.

It is evident that a cloud from a sprayer which throws comparatively large droplets will diffuse less widely, and will also tend to drop more rapidly, from the air of the room, than a cloud composed of finer particles. Under such conditions, the duration and extent of the killing action of the former will be

less than that of the latter, more especially in the case of insects, which are hidden and so sheltered from the direct action of the sprayer. At the same time, the quantity of solution needed to obtain a good result will be more, and the cost of effective spraying greater.

As will be seen in some of our experiments, there is a tendency for the toxic vapour to drop towards the floor of the room, and cause a more deadly effect upon insects in this position. Such a subsidence would be more rapid in the case of a cloud of large droplets than in one of smaller ones, so that many insects hidden in the higher parts of the room might escape, or merely be stupefied to recover later.

Gnadinger (1933) states that the relative toxicity of the oil used is more or less independent of its physical constants. While this may be so, it is probable that if a very volatile vehicle be used for the pyrethrum extract, the cloud obtained will be mainly volatile in character rather than composed of minute droplets. It is upon the carriage of the toxic element of the pyrethrum in such droplets that the action of the insecticide appears to depend mainly. If this be so, it would appear probable that an excessively volatile vehicle would not be so effective, apart from any other disadvantages. It has also been found that if the base oil is too heavy the killing power of the pyrethrum will be considerably reduced.

Recently the employment of atomizers worked by compressed air have been recommended for use in spacious buildings. Such sprayers would probably be very useful in increasing the diffusion of the cloud and the rapidity of its application. Gnadinger (1933) reports that steam-sprayers for this purpose are now attracting attention, and that several have been placed on the market. As will be seen from the next section, and from our experiments, the question of the effect of the high humidity produced by such steam sprayers upon the toxicity of the insecticidal solutions will have to be investigated further, before they can be recommended for general use.

(c) METEOROLOGICAL CONDITIONS.

In the case of pyrethrum sprays used to protect live stock by their repellent action, it is found that their efficacy varies with temperature and humidity, factors which influence the activity and vitality of the flies, as well as the volatility of the repellent.

Hartzell and Wilcoxon (1932) and Fleming (1933) have produced evidence that, in the case of some agricultural insect pests sprayed with pyrethrum, the factors involved in causing death and bringing about recovery after stupefaction are accelerated by an increase of temperature. If such insects get a sub-lethal dose, recovery is more rapid, but if the dose be lethal, death occurs more quickly at a higher temperature.

Michel (1935) found that his pyrethrum insecticide was most effective at about 85°F. against mosquitoes, and that the killing power diminished in proportion to the temperature. In the Peet-Grady test for the relative toxicity of insecticides against house-flies, emphasis is placed upon the temperatures at which the experiments are carried out (*vide infra*).

In the latter test, a standard of 60 to 70 per cent of relative humidity is laid down as one of the conditions needed. As will be seen from our experiments, there is much evidence to show that, with a rise in relative humidity to

a certain level, there is a fall in the efficacy of our insecticidal solution, while with a rise in temperature this is increased.

TESTING THE TOXICITY OF INSECTICIDAL SPRAYING MIXTURES.

Various methods for testing the efficacy of insecticidal sprays have been devised. Many of these have been described in detail by Gnadinger (1933). As a result of this work it has gradually become evident that the apparent toxicity of such solutions may vary with the vitality of the insects used, the type of sprayer employed, and the temperature and humidity at which the experiments were conducted, apart from the concentration of the toxic substance in the mixture.

In testing the value of such spraying solutions, the Peet-Grady method appears to be the one most often employed. In this method the solution is distributed through a special type of De Vilbiss spray, operated at a constant air pressure. The cloud is allowed to act upon the flies, which are contained in a special box, exposed to the vapour for a given time, and then removed to a normal atmosphere for observation. In these experiments, the 'knock-down' after 10 minutes is noted, but the toxicity is calculated on the percentage of dead flies found after 24 hours.

The National Insecticide Manufacturers' Association of the United States of America adopted this method of test in the preparation of standards of the value of different insecticidal sprays against house-flies. This body laid down, as a minimum standard for a general household liquid spray insecticide, that, (a) 95 per cent of flies should be knocked down in 10 minutes, (b) at least 60 per cent dead after 24 hours, (c) the petroleum insecticide base should not exceed 48.9°C. (120°F.) flash-point, as determined by the Tagliabue open cup method, (d) the tests should be conducted at 29.5°C. (85°F.) and 60 to 70 per cent relative humidity, and (e) the flies used in the test should be 5 days old.

These standards have recently been criticised by Gothard (1932), who considers (a) the temperature recommended is too high, as this is above the average in ordinary households (in the United States), (b) the size of the droplets produced by two sprayers of the same make may differ, and (c) the resistance of the flies raised in different laboratories may also vary considerably. These criticisms appear justifiable and, under these circumstances, the results obtained in one laboratory may not be comparable with those reported from another. It is, however, possible to compare the *relative* efficacy of samples of different insecticidal solutions, if these be tested in the same laboratory under identical conditions of apparatus, insects, temperature, etc.

The Peet-Grady method, after making due allowance for the points mentioned, appears eminently suitable for testing the efficacy of different liquid insecticides against house-flies. The method has, however, one drawback in tests against mosquitoes, and that is that the insects are admitted into the test-chamber *before* the solution is sprayed into it.

In nature, house-flies are mainly seen in exposed situations, and thus their destruction by household spraying methods is caused chiefly by the 'direct-hit' action of the insecticide. On the other hand, very many mosquitoes may be hidden in dark corners, nooks and crannies, so that they either pass unnoticed, or the direct action of the spray cannot be applied to them effectively. It is

essential, therefore, that in testing the value of any insecticide against adult mosquitoes, the method used should be a test rather of the indirect action of the spray, through its diffusion, than of its 'direct-hit' effect. It is known that insects may be killed by drenching them with solutions of low insecticidal properties, and although such solutions may be of value against flies, they would be of little value for general use against mosquitoes.

Some workers have tried to avoid this 'direct-hit' action, when testing the value of spraying solutions against mosquitoes, but in many cases the techniques used do not appear to have excluded this effect entirely. In the experiments about to be described, the insecticide was sprayed into the test-chamber *before* the mosquitoes were introduced, so in this way any question of the 'direct-hit' action could be excluded, and the results evaluated almost entirely upon the diffusible qualities of the insecticide.

METHODS USED IN OUR EXPERIMENTAL WORK.

Our preliminary experiments were carried out in the laboratories of the Malaria Survey of India, Kasauli, and later a large series of controlled tests were conducted at the Ross Field Experimental Station for Malaria, Karnal.

(1) APPARATUS AND TECHNIQUE USED FOR TESTING THE TOXICITY OF INSECTICIDAL SOLUTIONS AGAINST ADULT ANOPHELINES.

The method used by us for testing the relative toxicity of different insecticidal solutions was very similar to the Peet-Grady method employed in testing the value of such preparations against house-flies. When our original investigations were commenced we were not aware of the existence of the latter method, and our technique differs from it in certain details.

(a) APPARATUS EMPLOYED.

(i) *The testing chamber.*—This is a large rectangular box made of three plywood, and having a capacity of 45 cubic feet*. The interior is whitewashed to make it more nearly comparable to natural conditions in Indian bungalows. This washing was repeated at the end of each day's experiments†.

Three glass panels, two on one side and one on another, are present in the walls to allow of the observation of the insects during the tests, and also to enable one to take the readings of the thermometers which are placed inside the chamber.

The end walls, which are about 36 inches wide and 44 inches high, are hinged at the top, and so can be opened up completely to allow the chamber to be ventilated freely between experiments. In one of these end walls is another small door, about 18 inches wide by 32 inches high. This serves to admit the head and shoulders of the observer, when he is collecting any flying insects at the end of the experiment.

*In future work, it would probably be more convenient to have a chamber of a capacity of 50 cubic feet, as it would be easier to calculate from this figure the amount of insecticide needed per 1,000 cubic feet.

†In the Peet-Grady method, the inner surface of the walls of the testing chamber is coated with sodium silicate to make these non-absorbent. After each experiment the walls are wiped down with an absorbent cloth. It is probable that such a coating would be better than whitewash in future tests.

In the middle of each of the four walls, at about two-thirds of the distance from the floor, is a circular opening about 1 inch in diameter. These are used to admit the nozzle of the atomizer, and they are closed with corks when not in use.

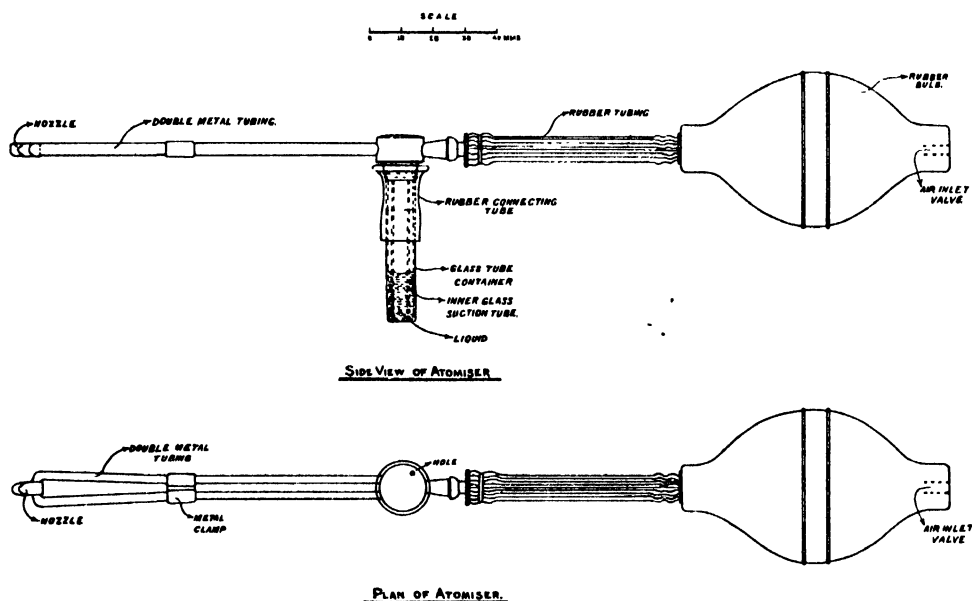
In the middle of the small door, there is another circular opening about $2\frac{1}{2}$ inches in diameter, through which the insects are admitted. This is closed by the lid of a cigarette tin.

The edges of the doors are covered with felt and fit tightly into the frame of the box, so that there is a minimum diffusion of the insecticide from the box during the experiments.

(ii) *The atomizer or sprayer* (text-fig.).—The instrument used is an atomizer made by the De Vilbiss Co. It is of the type commonly used as a throat spray, and is described by the makers as 'No. 15. Medium spray. Adjustable gold tip; for oils and aqueous solutions'.

TEXT-FIGURE.

Sketch of Atomiser.



The container of this instrument is much too large for use with the small quantities of insecticidal solutions being tested. It was therefore discarded, and replaced by a small glass specimen tube, about 1 mm. in diameter and 4 cm. long. This tube forms a convenient container for 1 c.c. of solution with little risk of overflow. It is also of such a depth that the intake tube of the atomizer is pressed firmly against its bottom when in position for spraying (*vide* text-fig.).

To make a tight connection between this tube and the atomizer, about $\frac{1}{8}$ to $\frac{1}{4}$ inch was cut off transversely from the rounded end of an ordinary

laboratory rubber teat. The flanged end, when adjusted over the screw by which the ordinary container is attached, grips it tightly. The wider cut end holds the mouth of the specimen tube, which is pushed into it until the end of the intake tube of the atomizer makes firm contact with its bottom (*vide* text-fig.).

(iii) *Amounts and strengths of spraying solutions.*—It was found that for the cubic capacity of the testing chamber, a quantity of about 0.2 c.c. of the best insecticidal solutions was sufficient to produce a killing action of about 100 per cent in 30 minutes. With the atomizer used by us, it was difficult to be certain that such a small quantity could be evenly distributed as droplets throughout the testing chamber. It was decided, therefore, that although 0.2 c.c. of the original solutions would be used in each experiment, this should be made up to 1 c.c. with an appropriate diluting fluid.

Practically all the insecticides used by us have some type of mineral oil as a basis for the solution of the toxic principle which they contain. It was necessary, therefore, to test whether the type of kerosene oil used in our work, had any appreciable toxic action on Anophelines when used alone under the conditions of our experiments. As we proposed to dilute the quantity of the original solution with 0.8 c.c. of this type of kerosene, we tried the effects of twice this amount (*i.e.*, 1.6 c.c.) sprayed into the chamber, and found no appreciable lethal effect was produced upon mosquitoes exposed for 30 minutes. This result confirms the statements of other workers that such oil has a very low toxic effect.

As a result of these experiments, we concluded that the dilution with kerosene of the insecticidal solutions to be tested, could have little effect on their relative toxicity. We, therefore, conducted our tests by placing 0.2 c.c. of the test solution in the container of the atomizer, and adding 0.8 c.c. kerosene to make a total amount of 1 c.c. in each experiment.

(iv) *The temperature and humidity in the testing chamber.*—A wet-bulb and a dry-bulb thermometer were placed inside the chamber, in such positions that they could be read with ease through the glass panels. The readings were made at the commencement of each experiment and again at the end. An average of these two readings was used in calculating the mean dry-bulb temperature and relative humidity under the experimental conditions in each instance.

(b) TEST INSECTS.

Because our interest as malariologists was chiefly concerned with female Anophelines, this type of mosquito was used for our work. There is some evidence to suggest that different species of mosquitoes may vary in their susceptibility to the toxic effects of insecticides, so our critical comparative experiments were all conducted with a single species, *A. annularis* (*A. fuliginosus*).

In our preliminary work at Kasauli, difficulty was experienced in obtaining locally sufficiently large numbers of a single species of *Anopheles*. In consequence, we had to get these from Karnal, whence they were sent to us by post in Barraud's boxes (*vide* Sinton, 1934). In this way a good supply of *A. annularis* and *A. subpictus* was available in our laboratory less than 24

hours after despatch. Although such insects appeared suitable for our preliminary tests, we considered that for more critical experiments the question of any loss of vitality, due to a journey under unnatural conditions, should be excluded. For this reason, our detailed experiments were conducted at Karnal, where very large numbers of adult Anophelines were available locally.

As it is very probable that there are considerable variations from day to day, in the vitality of adult mosquitoes bred under laboratory conditions from larvæ collected in nature, it was decided to conduct our experiments with wild insects caught locally. These insects were captured in test-tubes in cattle sheds, and kept in a large muslin cage under suitable conditions of humidity, until needed for experimental purposes. The majority of them were used within 24 hours after capture, and in very few instances were they used after 36 hours.

As the Anopheline most easily procurable at Karnal in large numbers during the whole course of our investigations was *A. annularis*, our experiments were conducted exclusively with this species, in order to ensure a uniformity in our conditions.

At the beginning of each day, a control set of 25 mosquitoes (*A. annularis*) was placed in a mosquito hotel, formed of a small hurricane-lamp globe*. A similar number of insects was used in each experiment carried out during the day. After exposure to the action of the insecticide, they were collected into hotels similar to those used for the controls, and stored under identical conditions. To ensure a favourable degree of humidity for the survival of these insects in the hotels, a small piece of damp lint was placed so as to cover partially the gauze cap at one end of the hotel, and a few moist raisins were also placed there to act as a food supply*.

(c) DETAILS OF TECHNIQUE.

(i) The interior of the testing chamber is re-whitewashed when the experimental work for any day is finished, sometimes more frequently if several different insecticides are being tested. The whitewash is allowed to dry before any new test is tried. The end doors are opened wide for at least an hour between each two experiments, to allow of proper ventilation. This ventilation was also facilitated by the fact that the apparatus was situated in a very large and lofty, open verandah and was thus exposed to any breeze blowing.

(ii) When the chamber had been thoroughly ventilated, the thermometers were placed in position and the doors closed tightly.

(iii) A carefully measured quantity of 0.2 c.c. of the test solution was placed in the container of the atomizer, and 0.8 c.c. of kerosene added, the whole being thoroughly mixed. The container was cautiously attached to the spraying apparatus. In the process, care was taken that none of the mixture was displaced out of it, and also that the intake tube of the atomizer was pressed firmly against its bottom.

(iv) The cork was removed from one of the small holes, the nozzle of the atomizer inserted inside the chamber, and two full blows of the atomizer were made by firm and complete compression of the bulb. The nozzle was removed and the cork replaced. This procedure was repeated in rotation at each small hole until all the solution had been sprayed into the chamber.

* A more detailed description of these 'hotels' is given by Sinton (1934).

(v) Twenty-five female specimens of *A. annularis* were then liberated into the chamber, and the effects of the insecticide noted. At the same time the readings of the thermometers were taken, and this was also done at the end of the experiment.

(vi) After 30 minutes had elapsed, the small door at the end of the chamber was opened, care being taken that no insect escaped. All the insects, both those flying and those recumbent on the floor, were collected, and counted to see that none had been lost. At the same time the number of those able to fly was recorded. These insects were placed in a mosquito hotel, as described above, and placed in a dark room for 24 hours, under the same conditions as the control insects.

(d) METHOD OF RECORDING RESULTS.

The end results of the experiments were recorded after 24 hours. Although with some of the less efficient insecticides, a number of the mosquitoes might still be flying after 30 minutes' exposure in the testing chamber, this was never the case with the more toxic solutions.

In recording the results, all those insects which were able to fly were classified as 'flying', those from which no active movement could be elicited when disturbed vigorously with a pair of forceps were noted as 'dead', while those that showed under such treatment some vital movement, however slight, of any appendage were considered to be 'moribund'. After the continued observation of a large number of such 'moribund' insects, it was found that none of them recovered their flying powers even after 48 hours. Under these circumstances, we decided to use as our index of toxicity for any insecticide tested, the percentage of mosquitoes which were 'dead' or 'moribund' at the end of 24 hours, as the latter, although still showing a spark of life, could be of little or no practical importance in the spread of disease, or as a nuisance.

The condition of the control insects was recorded in a similar manner. If these showed a mortality of more than 2 in 25, the results of that test were discarded.

(e) POSSIBLE FALLACIES IN TESTING EXPERIMENTS.

After having studied with care the results of our investigations, it was clear that if a definite *standard* test, in contradistinction to the comparative one used by us, was to be recommended for general use in testing the value of insecticides against mosquitoes, some modifications would be needed to exclude certain extrinsic factors.

In our experiments with the more promising insecticides, these fallacies have been eliminated to a large extent by the number of experiments carried out, but if tests are to be conducted as routine measures, it would not usually be practicable to make such a large series of tests for these determinations in each case.

(i) *Temperature and relative humidity*.—It has been found by us (*vide infra*) that the toxicity of some insecticides may be affected by variations in these conditions. In any standard test, arrangements should be made to keep these factors within certain narrow limits under test conditions, or alternatively any

comparison between two insecticides should be made under approximately the same conditions in the testing chamber.

(ii) *The type of atomizer.*—As has been found in the Peet-Grady method, this may have a distinct influence upon the results obtained. It is necessary, therefore, that *the same instrument* (not merely the same pattern of atomizer) should be used in the tests being compared. It is also necessary to see that before and during each test the instrument is working properly, and that there is no obstruction to the spray.

(iii) *The insects.*—These should be taken from the same population, and it would probably be better to use at least 50 insects in each experiment. The same number should be kept as controls. In any experiment where there is a high mortality among the control insects, the results should not be considered as admissible. In our experiments, if, after 24 hours, more than 2 insects out of 25 were unable to fly among the controls, the results of that test were discarded.

(iv) *The number of experiments.*—In testing the precise value of any insecticide, at least five tests should be made, and an average taken of the findings. These should always be compared with the results obtained from experiments with an insecticide of proved utility, tested under similar conditions, and carried out with insects taken from the same population at the same time.

(2) RESULTS OF EXPERIMENTS CARRIED OUT IN THE TESTING CHAMBER.

A large number of different insecticidal sprays, both locally prepared and proprietary, were tested by the method described by us, to determine their comparative values in the destruction of mosquitoes. A series of preliminary experiments were first made with these solutions, and, in the case of those in which a low mosquitocidal power was found, no further tests were conducted. To confirm the value of those insecticides which appeared to show a relatively high degree of efficacy, a further series of experiments were carried out, under more carefully controlled conditions.

(a) THE RELATIVE TOXICITY OF VARIOUS LOCALLY-PREPARED SPRAYS.

The usual ingredients recommended for such spraying solutions are carbon-tetrachloride or naphthalene dissolved in kerosene oil. Such insecticides were tested, either alone or in combination, but, when any question of the 'direct-hit' action was excluded, we were unable to obtain a toxic efficiency above about 16 per cent. Experiments were also made with kerosene-oil solutions of camphor (1 per cent), thymol (2 per cent), oil of wintergreen (2 per cent), oil of eucalyptus (5 per cent) and several other substances. In none of these was a maximum toxicity of over 30 per cent found, and in some instances it was as low as 4 per cent.

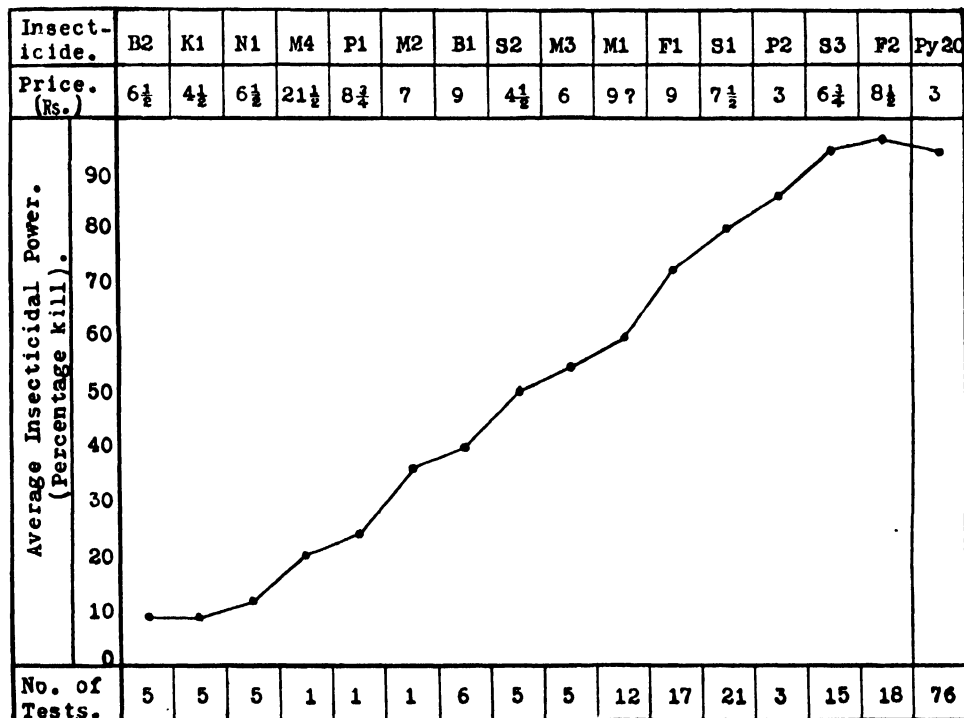
The chloroform extract of pyrethrum dissolved in kerosene, which was recommended by Holt and Kintner (1932), was also tried. We found that, as prepared locally, the efficacy was only about 20 per cent under the conditions of our experiment. This was possibly due to the type of pyrethrum flowers available. Even then, its cost was found to be little less than that of some of the more effective proprietary sprays.

(b) THE RELATIVE TOXICITY OF SOME PROPRIETARY INSECTICIDES.

A large number of the different insecticidal sprays available on the market in India were tested. The names of these have not been given, but are indicated by a letter and number (*vide* Chart I).

CHART I.

The relative toxicity of different insecticidal spraying mixtures.



(i) *Insecticide*—B*** (B1). Six experiments were carried out with this preparation. The results of these showed an average lethal rate of 40 per cent, and were in no case more than 64 per cent.

(ii) *Insecticide*—B***** (B2). This was tested on five occasions. The results gave an average death rate of 8·8 per cent, with a maximum of 24 per cent.

(iii) *Insecticide*—F*** (F1). As two preliminary experiments showed a death rate of about 78 per cent, a further series of 17 experiments were made. The latter gave an average killing power of 72·5 per cent, with a maximum of 100 per cent on two occasions.

(iv) *Insecticide*—F**** (F2). Eighteen tests were made with this solution, which showed an average toxic percentage of 96·4, with variations between 92 and 100.

(v) *Insecticide*—K** (K1). An insecticidal average of 8.8 per cent was found in five tests with this preparation, the maximum being 16 per cent.

(vi) *Insecticide*—M***** (M1). In twelve experiments conducted with this spraying solution, the lethal rate averaged 60 per cent with a maximum of 84 per cent in two of the tests.

(vii) *Insecticide*—M***** (M2). Only one experiment was made with this preparation, and the recorded lethal rate was 36 per cent.

(viii) *Insecticide*—M***** (M3). This solution was tested five times, and showed an average toxicity of 54.4 per cent, with a maximum of 80 per cent on one occasion.

(ix) *Insecticide*—M***** (M4). The one test made with this preparation gave a killing power of 20 per cent.

(x) *Insecticide*—N***** (N1). Five tests were conducted, in which the average lethal effect was 12 per cent. On one occasion it was as high as 32 per cent.

(xi) *Insecticide*—P***** (P1). This spraying solution was tried once and the toxicity was found to be 24 per cent. Another experiment, in which five times the amount of solution was used, only gave a toxicity of 68 per cent.

(xii) *Insecticide*—P***** (P2). This is a commercial extract of pyrethrum, which the makers recommend should be diluted 64 times with kerosene oil before use. The lethal rate in such a solution in three tests averaged 86.6 per cent, with a maximum of 92 per cent.

(xiii) *Insecticide*—S***** (S1). In four preliminary tests the killing power varied between 88 and 100 per cent. More careful experiments were made on 21 other occasions, and these gave an average toxicity of 79.2 per cent, with a maximum of 100 per cent in three tests.

(xiv) *Insecticide*—S***** (S2). The average toxicity found in five experiments was 49.6 per cent, with a maximum of 64 per cent.

(xv) *Insecticide*—S***** (S3). In three preliminary tests the toxicity varied between 80 and 100 per cent. A further series of 15 tests was made, in which the average was 94.4 per cent, with a maximum of 100 per cent on nine occasions.

(c) THE RELATIVE TOXICITY OF AN INSECTICIDAL SOLUTION MADE WITH PYROCIDE 20.

'Pyrocide 20' is a standardised concentrated extract of pyrethrum flowers prepared by the McLaughlin Gormley King Co., Minneapolis, Minnesota, U. S. A.*. The makers recommend that this extract be diluted for use in the proportion of one part to nineteen parts of refined kerosene. We have found that the brands of burning oil sold in Indian markets, under the names of 'Horse', 'Rising Sun' and 'Elephant', formed suitable diluents. The two former brands are said by the makers to meet the requirements of a 'non-staining, completely volatile kerosene with a flash-point not lower than 120°F. when taken by the official Tagliabue cup method'.

In a series of eight preliminary experiments, an average lethal rate of nearly 100 per cent was obtained. The preparation was, therefore, submitted to a large number of more critical tests.

*The agents in India for this concentrated pyrethrum extract are—Messrs. F. S. Kerr & Co., Vulcan House, Ballard Estate, Bombay. The present market price is quoted Rs. 35 per gallon.

The average toxicity of the spraying solution was found to be 93·8 per cent in a series of 76 controlled experiments, under varying conditions of temperature and humidity (*vide infra*). In 55 of these tests the lethal rate was more than 95 per cent, and in only five was it below 80 per cent. As can be seen from the results recorded above, and from Chart I, the efficacy of this preparation compares very favourably with that of the best proprietary sprays.

(d) DISCUSSION OF RESULTS OBTAINED IN THE TESTING CHAMBER.

(i) *Relative toxicity of different insecticides.*

None of the locally-prepared sprays, in which pyrethrum did not form an ingredient, were found satisfactory as insecticides, when the 'direct-hit' effect was excluded. This finding agrees with the opinions expressed by several other workers, who think that, in our present state of knowledge, only those insecticides which contain pyrethrum combine a high degree of efficacy with suitability for household use.

It is probable that the majority, if not all, of the best proprietary insecticidal sprays on the market contain pyrethrum in larger or smaller proportions. The average results of our tests of 15 different proprietary preparations are shown graphically in Chart I. From this and the results reported above, it is seen that in only two (Nos. S3 and F2) did we obtain an average lethal action of over 90 per cent, while in many others a very low toxicity was found, when, under the conditions of our experiments, the 'direct-hit' effect was excluded.

These two efficacious sprays were found to have an average toxicity which was very similar to that obtained with the pyroicide-kerosene solution. These results go to show that, as judged by our methods of testing, the lethal action of a mixture of one part of Pyroicide 20 with nineteen parts of kerosene oil is approximately equal to that of the best insecticidal spraying solutions on the Indian market at present.

(ii) *The effects of meteorological conditions on the toxicity of pyrethrum sprays.*

As has been noted earlier in this article, several workers have produced evidence to show that the relative toxicity of pyrethrum sprays may vary with changing conditions of temperature. In the Peet-Grady method, a definite temperature and range of relative humidity are laid down for the testing conditions.

In our work, a careful record was kept of the wet-bulb and dry-bulb temperatures inside the testing chamber, and from these the relative humidities were calculated. In the long series of tests carried out with Pyroicide 20 solution, these factors were found to have a distinct influence upon the lethality of the spraying solution.

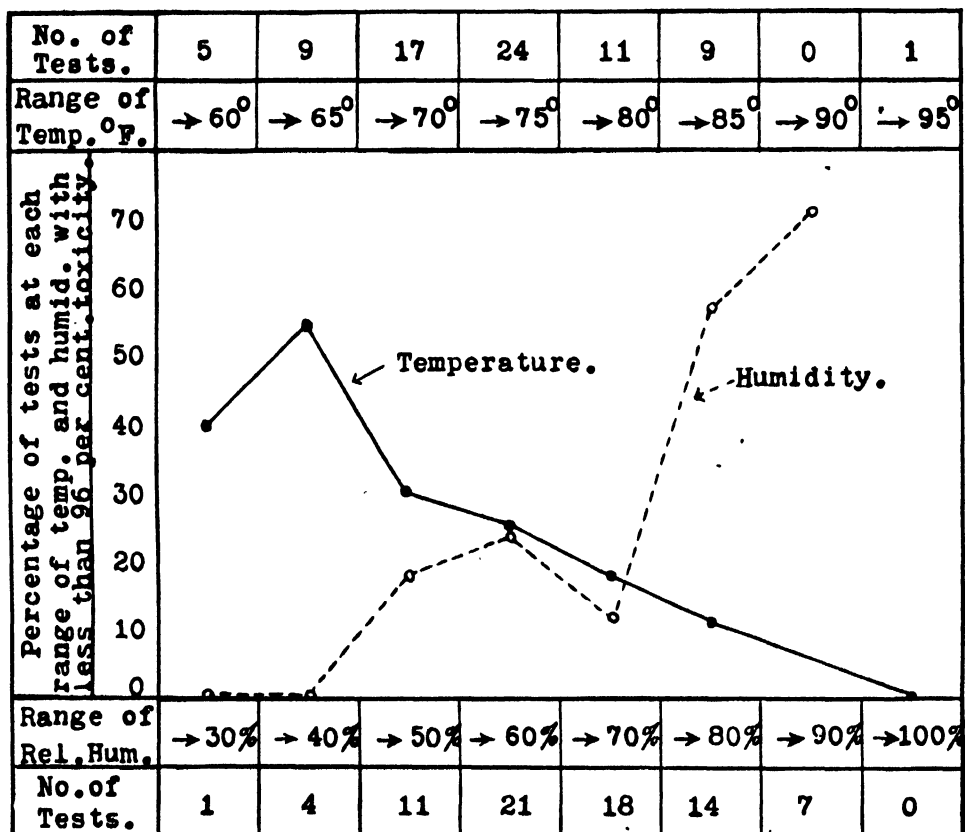
Our experiments at the Ross Field Experimental Station, Karnal, were conducted from the middle of March till the end of April, in an open verandah facing west. During this period, there were very great changes in the ranges of temperature and relative humidity in our testing chamber. Apart from the usual variations in the meteorological conditions which occur during this season, these factors were also influenced by the fact that many of the experiments were made in the early morning when the temperature was low, and others in

the afternoons, when the sunshine on the western verandah caused a marked rise.

The relative humidity in the experiments varied from 21 to 88 per cent, and the temperature between 58° and 98°F. The results of 76 tests of the Pyroside solution are shown graphically in Chart II. In this the temperatures are shown in ranges of 5° from 56° to 95°F., and the relative humidity in ranges of 10 per cent from 20 to 100 per cent. For each range of temperature and humidity, the percentage of the experiments which showed a lethal percentage of *less than 95*, at the different ranges, is charted.

CHART II.

The lowered lethality of pyroside-oil mixtures at different ranges of temperature and humidity.



The chart shows that, under the conditions of our experiments, the toxic efficacy of the insecticide rose as the temperature increased from 56° to 95°F. On the other hand, as the relative humidity rose from 20 to 90 per cent the efficacy of the solution decreased.

It is impossible to say, from the evidence available, what was the exact cause of the temperature effect. It may have been due to an increased activity

of the insects, and so more rapid and certain death from an increased intake of insecticide into the respiratory system*; or it may be that at higher temperatures the toxic principles in the droplets of the cloud penetrate the integument better; or the better result may be due to physical factors connected with the size of the droplets formed by the atomizer, and the extent of diffusion and rate of duration of the cloud in space.

The decreased efficiency observed with rising humidity is possibly associated with physical changes in the nature of a cloud of insecticide in an atmosphere already highly charged with aqueous vapour.

The results obtained in our experiments tend to confirm the opinion that meteorological conditions may have a considerable effect in influencing the relative efficacy of pyrethrum spraying solutions. The lethal action of such sprays appears to be increased by a rise in temperature above a certain point, and to decrease with a rise in relative humidity.

(3) RESULTS OF TRIALS OF PYRETHRUM-OIL SPRAYS IN ROOMS.

The results obtained by trials of pyrethrum-oil solutions in the small testing chamber were very encouraging. It was necessary, however, to ascertain whether this preparation would be of equal value when used in the larger spaces to be dealt with in the household application of such insecticides.

(a) TECHNIQUE.

(i) *Rooms*.—The preliminary experiments were made in a laboratory room at Kasauli. This room had a capacity of 2,410 cubic feet, with dimensions approximately—height 12 feet, length $16\frac{1}{4}$ feet, and width 12 feet. The more critical work was done at Karnal in a room of a capacity of about 3,910 cubic feet, the approximate measurements being—height 17 feet, length 16 feet and width 14 feet. The former room had three windows, two doors and a chimney, while the latter had two windows and a door, but no chimney. Beyond closing the doors and windows, no special precautions were taken to make the rooms air-tight.

(ii) *Distribution of the mixture*.—A 'Mysto' hand sprayer was used for this purpose. This is of the same type as the usual insecticidal spraying 'gun' employed for the household application of the common proprietary preparations.

Before the experiments were started, the 'gun' was tested to determine approximately how many *full* strokes of the instrument were equivalent to the discharge of a definite amount of solution. In each experiment, the amount was also checked accurately by placing a known quantity in the container of the 'gun', and measuring the amount left at the end of the experiment.

Unless otherwise stated, efforts were made to obtain an even distribution of the spray cloud to all parts and levels of the room. In distributing the solution, the operator walked systematically around the room spraying at different levels and in different directions in rotation. By calculating approximately the number of strokes of the gun needed to distribute the required

* Wigglesworth (1934) says 'normally, the spiracles are kept closed; being opened only just often enough to keep the insect supplied with oxygen. But when the insect is active, or when the rate of metabolism is increased through a rise in temperature, they are widely dilated'.

amount of the solution, and dividing this number into appropriate parts, a fairly even distribution can be obtained in different positions in the room.

(iii) *Test insects*.—In some of the Kasauli experiments, both *A. annularis* and *A. subpictus* were used, mainly the former, but at Karnal, *A. annularis* alone was employed.

These mosquitoes were enclosed in small cages, six inches square and made of fine mosquito netting. The same number of insects (usually 20) were placed in each cage, and an equal number from the same population were kept in a similar cage, as a control on the results of each experiment.

The insects were easily observed inside the cages, and the effects of the insecticide could be studied at different intervals. When the time of the experiment had expired, the cages were removed rapidly to the open air, and the results of insecticide recorded. The insects in their cages were then stored in a dark room under the same conditions as the controls. A piece of moist lint and a few damp raisins were placed on the top of each cage. The results of the experiment were again recorded after 24 hours, and the same criteria of lethal effect were used as with the trials made in the testing chamber.

(iv) *Position of cages in the room*.—While the room was being sprayed, the test insects were kept in the cages, which were placed inside a tightly closed cupboard until the process was finished. In this way any 'direct-hit' effect was eliminated.

When the insects were exposed to the effects of the solution, the cages were suspended in the corners of the room, so that one was at floor level, one at one-third the distance above this, one at two-thirds, and the upper one at the ceiling. This was done to estimate the lethal rate of the insecticide at different levels in the room.

In order that the cages could be placed quickly in position after the spraying was finished, they were each attached at appropriate positions to a long cord, the end of which was passed over a hook, one in each corner of the ceiling. This cord was of such a length that it was possible to place the cages inside the cupboard without detaching them from it, or displacing it from its attachment to the ceiling hook. It was thus possible to draw the cages into position within a few seconds after the spraying operation was finished.

In the preliminary experiments at Kasauli, only four cages were used, which were hung at appropriate heights in one corner of the room. In the Karnal experiments, twelve cages were employed in each experiment. Four spaced cages were hung at two diagonally opposite corners of the room, so making two cages at each of the four different levels. In another corner, one cage was placed near the ceiling, and one in the intermediate position immediately below this, while, in the other corner, one cage was at the floor level and one in the position immediately above this. In this way there were three cages at each of the four different levels in the room, and the results in these positions were recorded separately (*vide* Tables I and II).

(b) LETHAL EFFECTS OF PYROCIDE-OIL SOLUTIONS.

The trials made in the testing chamber showed that such solutions produced a very high lethal action, when used in the proportion of 1 c.c. per 225 cubic feet, under our experimental conditions.

Three trials were then made in a room at Kasauli, without blocking the chimney. The solution was used in a strength of 1 c.c. per 193 cubic feet. An average lethal rate of about 78 per cent was obtained among about 100 mosquitoes in each experiment, although the temperature at the time was low, being about 60°F. or less. When the chimney was blocked to prevent much loss of strength of the insecticidal cloud, the percentage of kills rose to 90.

Another series of nine more carefully controlled tests were then made at Karnal. As will be seen from the results recorded in Table I, the average lethal rate was nearly 97 per cent, with varying strengths of the solution and times of exposure. The immediate effects as judged at the end of exposure were good, for, except for one insect in experiment No. 2, one in experiment No. 9, and three in experiment No. 7, all the mosquitoes were moribund.

One experiment was made with a well-known proprietary insecticide (F1). Five insects were still able to fly at the end of the test, and the final lethal rate found was only about 63 per cent.

The results recorded help to confirm the opinion that the pyroicide-kerosene-oil solution has a comparatively high degree of efficacy against mosquitoes, when tried under the ordinary conditions of household use.

TABLE I.

Summary of the results of spraying pyroicide-oil mixture in a room.

Experiment number.	Temperature (dry-bulb °F.).	Relative humidity (per cent).	Concentration of spray—1 c.c. per—cubic feet space.	Duration of exposure to spray (minutes).	Concentration ÷ exposure.	Total number of Anophelines.	PERCENTAGE LETHAL RATE AT FOLLOWING POSITIONS IN ROOM—				Total lethal rate (per cent).
							1 (top)	2	3	4 (floor)	
1	63½	60	130	10	13	240	100	100	98.5	100	99.5
2	61	55	206	10	20	240	95.0	96.6	95.0	98.3	96.2
3	59	67	112	15	8	120	96.6	100	100	100	99.2
4	60	51	122	15	8	180	88.8	97.7	100	97.7	96.2
5	59	48	186	15	12	240	98.3	100	98.3	100	99.2
6	60	71	200	15	13	240	96.6	100	98.3	98.3	98.3
7	59	48	390	15	26	240	88.3	98.3	98.3	90.0	93.7
8	57	13	356	20	18	240	83.0	96.6	98.3	98.3	94.2
9	58	54	373	30	12	240	93.3	95.0	98.3	96.6	95.8
Average of nine experiments							93.3	98.0	98.0	97.5	96.8
F1	56	45	187	15	12	156	35.9	64.1	76.9	74.1	62.8

(c) THE RELATIONSHIP OF LETHAL EFFECTS TO THE STRENGTH OF, AND THE DURATION OF EXPOSURE TO, PYROCIDE-OIL SOLUTIONS.

The makers of proprietary preparations do not appear to lay down any precise details as to the amounts of these solutions to be used in a given space to produce an effective insecticidal action. Replies received from various sources have been indefinite, or tentative estimates of amounts from 2 to 8 ounces per 1,000 cubic feet have been suggested. Michel (1935) reports that a 4 per cent mixture of Pyroicide 40 was efficacious against *Aedes aegypti* in aeroplanes, when used in a concentration of 200 c.c. per 3,000 cubic feet for 15 minutes.

The strength used in the testing chamber was 1 c.c. per 225 cubic feet, and this gave a high lethal effect with an exposure of 30 minutes. As will be noted later, this concentration in a closed room gives after a time a slightly oppressive feeling to the occupants, and, in some cases, a more rapid action than 30 minutes' duration may be desirable.

As will be seen from Table I, various concentrations of solution, and durations of exposure were tested. It was found from observations made at the end of each experiment that a larger number of the insects were able to fly, or showed active movements, when a weak cloud was used than when a more concentrated one was tested, even although the duration of exposure was proportionately longer. The findings after 24 hours also suggest that a higher final lethal effect is produced by a greater strength of insecticide acting over a short time, than by a proportionately weaker one acting over a longer period. In both instances, however, the lethal rate was over 90 per cent. To obtain a more precise correlation between the concentration and time factors, a larger number of carefully controlled experiments are needed under similar conditions of temperature and humidity.

Some observations were made which suggested that the cloud produced by the De Vilbiss atomizer was composed of finer particles than those formed by the ordinary commercial 'gun'. Under these conditions, the cloud produced with the latter type of sprayer would probably be less lasting and lethal.

It is interesting to note that in three trials with the pyroicide solution and one with a proprietary preparation (No. F1), a few Culicine mosquitoes (*C. fatigans*) were seen free inside the room at Karnal, while the experiments were in progress. These insects appeared to have a greater resistance to the cloud of insecticide than had the Anophelines, and the males seemed much more resistant than the females. This greater resistance of Culicines, as compared with Anophelines, was noted by Brug and Van Slooten (1927). Michel (1935) also used much larger concentrations of his spraying mixture to disinfest aeroplanes of *Aedes aegypti*, than we have found necessary in our experiments with Anophelines.

As the mosquitoes in our experiments were enclosed within cages of fine gauze, it is possible that the diffusion of the cloud to the insects was hindered to a certain extent by the obstruction afforded by the netting. It may be, therefore, that under more natural conditions an even higher lethal rate would be obtained than that reported in our work. On the other hand, the process of collection and storage of our test insects may have lowered their vitality to some extent, although this was not indicated by the controls kept.

(d) EFFECTS OF THE DIFFUSION AND DILUTION OF PYRETHRUM SPRAYS ON THEIR LETHAL ACTION.

As noted previously, the cages containing the test insects were hung at various heights in the rooms where the efficacy of the pyroicide solutions was being tried. Some cages were placed at floor level, and some close to the ceiling, while others were suspended in intermediate positions, one-third to two-thirds the height of the room above the floor.

In three preliminary tests with pyroicide solutions at Kasauli, it was found that the relative killing efficacy was on an average 70·7 per cent at the ceiling, and only about 58·5 per cent at the floor level in a room with an open chimney. At the two intermediate positions, the average percentages were 90·7 (above) and 76·9 (below). This curious distribution, following an even distribution of the spraying, suggested that (i) the cloud of insecticide tends to drop floorwards, so reducing its toxic efficiency near the ceiling, and (ii) that at floor level and some feet above it, there was a large amount of diffusion or dilution of the cloud through the chimney opening.

Another test was made to investigate these suggestions. The chimney opening was blocked before this trial was made. Here the results obtained were 80 per cent near the ceiling and 90 per cent near the floor, with percentages of 90 (above) and 100 (below) in the intermediate positions. These findings help to support the view (i) that the insecticidal cloud tends to fall, and (ii) that there was probably a great diffusion or dilution of this cloud through the chimney opening, to account for the diminished toxicity reported in the different positions in the room. The detailed results of these trials are shown in Table II.

Two other trials were made with an open chimney in the room. In one of these the spraying was directed entirely towards the upper part of the room, and in the other just above floor level. The results also showed the highest killing percentage in the upper of the two intermediate positions in both cases.

A series of more carefully controlled experiments were made in a room at Karnal, to obtain confirmation of these results. As noted previously, this room contained no chimney, and the main openings, allowing of possible diffusion or dilution of the cloud, were under the door and through a waste-water exit, about 4 inches diameter, opening into the open air, near the floor in one corner. These openings were not occluded during the trials.

In these tests it was found that the mosquitoes in the cages near the floor were stupefied more rapidly than those in the higher cages, and that the insects in the cages near the ceiling continued to fly for the longest time.

The results of these later experiments with the pyroicide solution (*vide* Table II), when varying strengths and periods of exposure were used, showed (i) an average toxicity of 93·3 per cent at ceiling level, (ii) 98 per cent in each of the intermediate positions, and (iii) 97·5 per cent near the floor. It was in the last position that the diffusion and dilution was greatest through the opening under the door and that for waste water. These effects were, however, much less than those obtained with the open chimney in the preliminary experiments.

When one studied the percentage of insects which were alive after 24 hours in the lower cages near the door and the waste-water opening, it was found this was about 3·3 per cent as compared with 0·6 per cent in the corner away from these openings. Similarly in the lower intermediate cages in the two former

corners, the rate was 1·8 per cent. These results tend to show that diffusion and dilution of the toxic cloud was taking place through these small openings. The diminution in average toxicity was, however, small, but appeared sufficient to counteract the greater toxicity near the floor which would have been expected from the sinking of the cloud of insecticide.

Another experiment was made in which the spraying was all done directly towards the ceiling and the upper corners of the room. An electric table fan, of about 15 inches diameter, was placed in the middle of the floor, and directed towards the ceiling. As soon as the spraying was finished this was made to revolve at full speed. The results obtained were killing percentages of (a) 90 at the ceiling, (b) 100 in the upper intermediate position, (c) 93·3 in the lower one, and (d) 70 near the floor (*vide* Table II). The effect of the fan appeared to be that it prevented a high concentration of the insecticide near the floor, and probably at the same time was responsible for an influx of air into the lower portions of the room to dilute the toxic cloud. The latter suggestion is supported by the fact that, in this experiment the total lethal rate was only 88·3 per cent, as compared with an average of 96·8 per cent in the experiments without the fan.

In one experiment (No. 3, Table I) the observers left the room rapidly after the spraying was finished, and remained outside until the duration of exposure was over. This very rapid opening and closing of the door did not appear to have a marked effect upon the lethal action recorded.

Another controlled experiment, similar to the first ones, was made with a proprietary preparation (F1). In this trial the results obtained also showed a lower mortality among the test insects placed near the ceiling (*vide* Table II).

The findings recorded under the conditions of our experiments appear to show that pyrethrum-oil insecticides act less efficiently in the upper parts of a

TABLE II.
The lethal action of insecticides at different levels in rooms.

Place.	Insecticide.	Number of tests.	PERCENTAGE OF EFFICACY AT POSITIONS—				Total lethal rate (per cent)	REMARKS.
			1 (top)	2	3	4 (floor)		
Kasauli.	Py 20	3	70·7	90·7	76·9	58·5	74·2	Chimney open.
	Py 20	1	80·0	90·0	100	90·0	90·0	Chimney closed.
Karnal.	Py 20	9	93·3	98·0	98·0	97·5	96·8	No chimney in room.
	Py 20	1	90·0	100	93·3	70·0	88·3	No chimney; fan blowing towards ceiling.
	F1	1	35·9	64·1	76·9	74·1	62·8	No chimney in room.

room than in the lower. This is probably due to a rapid settling down of the toxic cloud. The results also show that there may be a distinct diminution in the insecticidal power of such sprays, at positions where there are opportunities for the dilution or diffusion of the cloud through openings in the room sprayed.

(e) UNPLEASANT EFFECTS CAUSED BY PYROCIDE SPRAYS.

In all the trials at Karnal, except one, the observers stayed in the room while the tests were in progress.

As the room used was a high one, when the spraying was being done towards the ceiling, small particles of the solution tended to fall into the eyes while the operator was looking upwards. This caused a tingling sensation, which passed off quickly in the open air.

When the pyroicide solution was used in concentrations of about 1 c.c. to 100—150 cubic feet, the occupants of the closed room felt in about 5 minutes a distinctly oppressive feeling, which developed into a mild headache, and sometimes a slight irritation of the nasal mucosa was also experienced.

In concentrations of about 1 c.c. per 200 cubic feet, except for a slight oppressive feeling, the symptoms were slight, while, in concentrations of about 1 c.c. per 350—400 cubic feet, very little unpleasant effect was noted in a closed room up to 30 minutes. When a fan was working in a room with a concentration of about 1 c.c. per 200 cubic feet, the effects were much less, probably due to the greater dilution and diffusion of the cloud.

In the test with the proprietary preparation (F1) in a strength of 1 c.c. per 156 cubic feet, the oppressive feeling was less than with the pyroicide solution, but the lethal action to the mosquitoes was also much less.

THE SUITABILITY OF A PYROCIDE-OIL MIXTURE FOR MOSQUITOCIDAL PURPOSES.

A solution of a concentrated extract of pyrethrum flowers (Pyroicide 20) in mineral oil has been found to be a very effective mosquitocidal spraying mixture. It is necessary to consider in how far this solution meets the requirements which we have suggested as suitable for an ideal preparation for this purpose.

(1) TOXICITY TO MOSQUITOES.

The solution has been found to have a very high toxicity to Anophelines, and this, under our experimental conditions, was equal to that of the best proprietary preparations tested. This toxic effect occurs in the absence of any 'direct-hit' action, thus making the mixture very suitable against mosquitoes.

The solution appears to be less effective against certain Culicines than against certain Anophelines. A similar result was, however, noted by us when a proprietary preparation (No. F1) was tested. It is therefore probable that a higher concentration than usual would be needed to destroy certain species of Culicines.

The range of temperature and relative humidity over which it gives a high lethal action appears to be wide. In this respect it falls somewhat short of

the ideal spray, but it is probably no less efficacious than the other pyrethrum spraying mixtures on the market.

(2) HARMLESSNESS TO MAN.

The mixture caused slight headache, an oppressive feeling, and some irritation of the nasal and conjunctival mucosa, when used in high concentration in a closed room. These unpleasant effects do not appear to be more marked than with other sprays of a similar degree of efficacy. In lower concentrations with a longer period of exposure, the lethal action is still high and the unpleasant effects are slight or absent.

Gnadinger (1933) states definitely that these pyrethrum-oil solutions are non-toxic to man. We observed nothing to suggest that in the strength used by us they had any toxic action.

The odour produced was that of kerosene oil, and quickly passed off. This odour could be masked by the addition of small amounts of oil of citronella, oil of sassafras, or oil of pine, as suggested above. The addition of such ingredients would probably add to the toxic action and usefulness of the mixture against mosquitoes.

The inflammability of the solution was tested. It was found to be inflammable when sprayed directly upon an open flame. When sprayed on a lighted cigarette end, or on a smouldering piece of lint, no flame was produced, and continued spraying tended to extinguish the burning point. When spilt on a wooden or a stone floor, attempts to light it with a match were unsuccessful. All these results were compared with those obtained with three of the well-known proprietary preparations (Nos. F1, S1 and S3), and in no case was the inflammability of the pyroicide-kerosene mixture found to be any greater than these.

When used in the ordinary concentrations, no damage appeared to occur to clothing, draperies or furniture.

(3) COST.

The market prices of various proprietary insecticides sold in India have been noted in Chart I. From this it will be seen that those insecticides which showed a high degree of efficacy against mosquitoes had a price at least twice as high as our locally-prepared mixture. The latter was made at about Rs. 3 per gallon.

(4) APPLICATION OF SPRAY.

The mixture was found to be applied easily in rooms, by means of a hand-sprayer similar to those used for proprietary preparations.

The results obtained show that a high toxic efficiency can be obtained, even when no elaborate precautions are taken to seal up rooms.

The rate of action with the higher concentrations has been found to be rapid, under the conditions of our experiments, while with lower concentrations a duration of exposure of about 15 to 20 minutes gave a high lethal percentage (Table I).

(5) SUMMARY.

These results go to show that an insecticidal mixture composed of one part of Pyroicide 20 in nineteen parts of kerosene oil will produce an insecticide which is equal in its mosquitocidal efficacy to the best proprietary sprays at present available on the market in India, and at about half the cost.

This spraying solution would probably be improved by the addition of either oil of citronella (5 per cent) or oil of sassafras ($\frac{1}{2}$ to 1 per cent) or oil of pine (4 to 5 per cent). These adjuvants would not only mask the odour of the kerosene oil, but would possibly increase its efficacy, and give it some repellent properties against mosquitoes.

The mixture should be stored in tins (old petrol tins are suitable), or in brown bottles, to prevent deterioration. As the solution is so easily and rapidly made in small amounts, the question of storage over long periods should seldom arise. The place of storage should be, as in the case of other pyrethrum-oil sprays, as cool and dark as possible.

In conclusion, it must be pointed out that our trials were made under laboratory conditions, and mainly with one species of *Anopheles*. It will be necessary, therefore, to determine whether the concentrations of Pyroicide 20 found effective by us in the laboratory will be equally so under natural conditions, and against other species of mosquito.

CONCLUSIONS.

From the results obtained under the conditions of our experiments, the following tentative conclusions appear justifiable—

(1) In testing the value of insecticidal solutions for the destruction of mosquitoes, the 'direct-hit' effect should be excluded.

(2) There are great differences in the relative toxicity of various insecticidal sprays on the market in India, as judged by the actions on mosquitoes. Some appear to be almost useless as mosquitocides, when the 'direct-hit' action is eliminated.

(3) Insecticides containing extracts of pyrethrum flowers appear to be more efficacious and suitable for household use than those mixtures which do not contain this substance.

(4) Concentrated extracts of pyrethrum flowers are being sold by commercial firms, and some of these extracts, when mixed with kerosene oil, can be used as effective insecticides against adult mosquitoes.

(5) A mixture of one part of such an extract (Pyroicide 20) with nineteen parts of kerosene oil has been found to be a comparatively cheap and highly efficacious mosquitocide for household use in India.

(6) The insecticidal efficacy of pyrethrum-oil spraying solutions appears to increase with a rise in temperature and to fall with a rise in relative humidity.

(7) Clouds produced by spraying such solutions tend to fall towards the floor, and so their efficacy is diminished in the upper parts of a room. Their relative toxicity is also diminished in those positions in the room where the concentration of the cloud is liable to dilution or diffusion.

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STUDIES IN IMMUNITY IN MALARIA.

Part IV.

THE RESULTS OF MULTIPLE HETEROLOGOUS SUPERINFECTIONS, WITH A DISCUSSION OF THEIR RELATIONSHIP TO SOME EPIDEMIOLOGICAL PROBLEMS AND TO THE GENERAL PRINCIPLES OF TREATMENT.

BY

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INTRODUCTION.

MULLIGAN and SINTON (1933*a*, 1933*b*), in two previous articles of this series, have recorded the effects of heterologous superinfections with different strains of *P. knowlesi*. These authors (Mulligan and Sinton, 1933*b*) concluded that, under the conditions of their experiments,—

(1) 'Multiple heterologous superinfections with certain strains of *P. knowlesi*, appear to produce a considerable degree of tolerance against some other heterologous strains of this parasite, but not against all strains'; and

(2) 'It appears very probable that, when multiple superinfections with several heterologous strains produce any considerable degree of tolerance to other heterologous strains, these strains have some immunological factors in common. In the absence of such common factors, multiple heterologous superinfections appear to produce no very effective tolerance'.

Mulligan and Sinton (1933*b*) have given the detailed histories of six monkeys, two of which received 3, three received 4, and one received 5 heterologous superinfections. We have continued these experiments with the five animals which survived, until the number of heterologous superinfections with *P. knowlesi* reached a total of 6 in each case. The results of these experiments are recorded here, and have been summarised in Table I, along with the earlier results reported by Mulligan and Sinton (1933*b*).

It was pointed out in our previous work that most of the original hosts (*S. irus*) of our strains, were suffering from mixed infections of *P. knowlesi* and *P. cynomolgi*. When such animals were used as donors for superinfections, chronic mixed infections with both species of *Plasmodium* have resulted in some instances. These observations help to confirm the conclusions reached by Sinton and Mulligan (1933), that the original infections in *S. irus* were mixed ones, and that the morphological appearances seen when infected blood was inoculated into *S. rhesus*, were due to such mixed infections in the latter host, and not to any change in the morphology of the parasite in a different species of host. The occurrence of such chronic mixed infections in *S. rhesus* was seen more commonly when a homologous superinfection had been made from the original host of a strain. Under these conditions, the results produced with the homologous strain of *P. knowlesi* were so mild that treatment was never necessary, so there was no chance that the infection with *P. cynomolgi* would be eliminated by the treatment usually given to control the infection with *P. knowlesi* (Sinton and Mulligan, 1933). It will be seen, therefore, that in several of our animals mixed infections with both species of *Plasmodium* have been recorded, due to the fact that in some of the previous superinfections the donor monkey was suffering from a mixed infection*.

METHODS AND TECHNIQUE.

The methods used were the same as those described by Mulligan and Sinton (1933b) in the previous work. The inoculations were made by intraperitoneal blood injections.

It was pointed out by Mulligan and Sinton (1933b) that a sufficient period must be allowed to elapse between superinfections to give the animal time to develop a tolerance to the strain last inoculated. In the present experiments, intervals of 2 to 3 months were allowed to elapse in each case. As will be seen from the protocols, no specific treatment was given in the superinfections, so the reported diminution in the development of immunity from this cause can be excluded in the superinfections here recorded.

The evaluation of the results of superinfection has been made in the same manner as described by Mulligan and Sinton (1933b). As the clinical manifestations in multiple superinfections are usually slight, we have depended mainly upon the changes produced in the numerical prevalence of parasites in the peripheral blood. These changes were determined by daily blood examinations by the thick-film method, continued for long periods after each superinfection was made.

THE EFFECTS OF MULTIPLE SUPERINFECTIONS WITH HETEROLOGOUS STRAINS OF *P. KNOWLESI*.

The effects of continued heterologous superinfections of four specimens of *S. rhesus* (Nos. 7, 29, 39 and 80) and one of *S. sinicus* (No. 82) are given below. In Table I is also included a summary of the history of *S. rhesus*, No. 9, which

*This appearance of a predominant infection of *P. cynomolgi*, at some stage of the infection in animals immunised against *P. knowlesi* and reinfected from another animal with a mixed infection, was suggested by Sinton and Mulligan (1933) as a means whereby the former species might be separated from the latter in mixed infections.

died at the 5th heterologous superinfection, and of which a detailed account has already been given by Mulligan and Sinton (1933b). The numbers given to the experiments are the same as those used by the latter workers, so that reference may be facilitated.

(v) Monkey No. 7 (*S. rhesus*).

The details of the histories of the primary infection, one homologous and four heterologous superinfections of *P. knowlesi* in this animal have been given in Experiment (v) of Mulligan and Sinton (1933b). The results of these are summarised in Table I of this paper. Since that time this monkey has been superinfected with two other heterologous strains of *P. knowlesi*. As Monkey No. 7 was under observation for some time after the period already recorded in the 4th* heterologous superinfection, the complete history of the latter is also given here.

History of primary infection and earlier superinfections. These are given by Mulligan and Sinton (1933b) (pp. 825-827).

History of 4th heterologous superinfection. Superinfected on 523rd day with strain K₁ of *P. knowlesi*. Blood taken from an old chronic superinfection in Monkey No. 110 (*S. rhesus*) (passages 2 and 3) [vide Experiment (f) of Mulligan and Sinton, 1933a]. Parasites† detected from 524th day onwards, with marked increase from 525th to 530th day, from 534th to 542nd day, and from 548th to 550th day, diminishing in intervals. A distinct parasitic relapse due to *P. knowlesi* occurred from 563rd to 573rd day. A marked parasitic attack of *P. cynomolgi*‡ developed between 580th and 588th day. After three days, during which both species of *Plasmodium* were found, an attack of *P. knowlesi* occurred lasting up to 606th day; parasites scanty. No parasites were found again until 618th day, when *P. knowlesi* again appeared to be replaced by a mixed infection of a slight nature. From 630th to 633rd days *P. knowlesi* alone was seen in scanty or very scanty numbers.

Result. A definite parasitic attack due to *P. knowlesi* developed without clinical symptoms. This was followed by several other distinct parasitic relapses, one of which was due to *P. cynomolgi*.

History of 5th heterologous superinfection. Superinfected on 633rd day by blood inoculation of strain K₁. Blood taken from Monkey No. 162 (*S. irus*) (original host) during very chronic infection. Parasites seen daily in very scanty numbers up to 647th day, and not detected again until 651st day. A distinct parasitic relapse then occurred with maximum on 654th and 655th days; infection again declined and none found from 659th to 661st day. Parasites again increased in numbers and were numerous on 663rd and 664th days. Later declined rapidly in number and were not found from 672nd to 691st day, except very few from 681st to 683rd day. A distinct parasitic relapse of *P. knowlesi* occurred between 692nd and 696th days, after which parasites found daily in scanty numbers until 715th day. No parasites found again until 734th day, when appeared in scanty numbers for 4 days.

Result. A short but definite parasitic attack without clinical symptoms, followed by a distinct parasitic relapse.

History of 6th heterologous superinfection. Superinfected on 738th day by blood inoculation with strain K₁ from Monkey No. 564 (*S. rhesus*) (passage 4); inoculated blood taken during an acute attack produced by splenectomy during the course of a chronic infection. No parasites detected after inoculation until 745th day, when

* This 4th heterologous superinfection corresponds to the 5th superinfection of Mulligan and Sinton (1933b), because this animal also received a homologous superinfection before any heterologous ones were tried.

† Unless otherwise specified in these histories of superinfections, the term 'parasites' may be taken to mean *P. knowlesi*.

‡ The donor animal used for the 3rd heterologous superinfection had a mixed infection of *P. knowlesi* and *P. cynomolgi*.

P. cynomolgi only found in scanty numbers for 3 days. A very scanty mixed infection of *P. cynomolgi* and *P. knowlesi* present on 748th and 749th days, after which extremely scanty infection of latter parasite alone found daily until 758th day. No parasites seen again until 768th day, except very few *P. knowlesi* on two occasions. From 768th to 780th day, *P. cynomolgi* only found; at first very scanty, but later more abundant. On 780th and 781st days both species of parasite seen in scanty numbers. From this day till 794th day only *P. knowlesi* in extremely scanty numbers; no parasites found for 3 days. From 797th to 808th day this parasite increased to scanty numbers and declined again. No parasites seen on 809th day, but on 810th an apparently pure infection of *P. cynomolgi* developed and continued in scanty numbers up to 819th day. Blood examination negative on 820th day, but from 821st till 847th, *P. knowlesi* found daily in scanty or very scanty numbers. No parasites found again until 868th day, except very few *P. knowlesi* on one occasion. A scanty mixed infection detected from 868th to 870th day, and then only *P. knowlesi* for 4 days. No parasites found from 875th till 894th day, except few latter parasites on one day. *P. cynomolgi* again appeared from 894th to 896th day, then a scanty mixed infection for 2 days. *P. knowlesi* alone found in scanty numbers from 899th to 903rd day; then gradually disappearing until blood free on 910th day, and remaining so till 935th, except for extremely few parasites on 3 isolated occasions. Parasites again detected in scanty numbers on 935th, 936th, 937th and 945th days; except for these occasions, no parasites found in observation period lasting till 954th day, when animal was treated in connection with another experiment.

Result. Although slight but distinct increases in the numbers of *P. knowlesi* were detected on several occasions, these did not seem sufficiently great to conclude that the superinfection had caused a definite attack. Relapses of *P. cynomolgi* and mixed infections were seen on several occasions. In these *P. cynomolgi* was usually detected first, but was soon replaced by *P. knowlesi*.

Summary of results of 5th and 6th heterologous superinfections in Experiment (v).

A heterologous superinfection of strain K_6 superimposed upon previous infections with 5 other strains of *P. knowlesi*, produced a short, but definite, parasitic relapse. When the same animal was then given a further heterologous superinfection with strain K_5 , this caused a slight, though distinct, increase in the numbers of *P. knowlesi*. A mixed infection with *P. cynomolgi* was seen on several occasions. This was derived from the original host of K_6 (*S. irus*) which had a mixed infection and from which the 5th heterologous superinfection was made. Several relapses due to the latter parasite were observed.

(vi) Monkey No. 29 (*S. rhesus*).

The details of the histories of the primary infection, one homologous and four heterologous superinfections of this animal have been given in Experiment (vi) of Mulligan and Sinton (1933b). Since that time this monkey has received two other heterologous superinfections, and the summarised history of these infections is shown in Table I.

History of primary infections and previous superinfections. These are given by Mulligan and Sinton (1933b) (pp. 827-829).

History of 4th heterologous superinfection.* Superinfected on 452nd day with strain K_1 . Blood taken from Monkey No. 110 (*S. rhesus*) (passages 2 and 3), during the course

* This 4th heterologous superinfection corresponds to the 5th superinfection of Mulligan and Sinton (1933b), because this animal also received a homologous superinfection before any heterologous ones were tried. The history of this superinfection has been given in full to bridge the interval between the observations recorded by the latter workers and the present ones.

of a chronic homologous superinfection. Parasites increased in number from 454th to 462nd day, then decreased again and were absent from 465th to 476th day. A distinct parasitic relapse due to *P. cynomolgi** then occurred, reaching a maximum about 484th day and gradually diminishing. A mixed infection of *P. knowlesi* and *P. cynomolgi* detected at most daily examinations from 490th to 496th day, but former species quickly predominated and caused a distinct parasitic relapse from this time up to 504th day. Parasites then rapidly diminished and were absent from 509th to 514th day after which a marked parasitic relapse of *P. cynomolgi* occurred and this parasite persisted till 532nd day. A mixed infection then found for 2 days and a distinct relapse of *P. knowlesi* from 535th to 544th day; followed by a very scanty mixed infection for 2 days. Another relapse of *P. cynomolgi* between 547th and 553th days; both species of parasite found in scanty numbers from 556th to 560th day, and then *P. knowlesi* alone for 2 days.

Result. Superinfection was followed by a distinct parasitic attack of *P. knowlesi*; later definite relapses of this parasite and of *P. cynomolgi* occurred.

History of 5th heterologous superinfection. Superinfected on 562nd day with strain K_a from Monkey No. 162 (*S. irus*) (original host); blood taken during the course of a very chronic infection.

P. knowlesi found in very scanty numbers for 2 days after inoculation; no parasites seen again till 570th day, when distinct relapse of this species developed lasting until 580th day with maximum on 573rd and 574th days. A mixed infection then appeared, developing into relapse of *P. cynomolgi* with its maximum from 585th to 589th day. Blood examinations negative from 591st till 611th day, when *P. knowlesi* found in scanty numbers for 3 days. No parasites seen from 614th to 616th day, after which a slight infection of *P. cynomolgi* lasting till 629th day, to be replaced by a very scanty infection of *P. knowlesi*, which continued till 635th day. From this time till 664th day no parasites found, except scanty *P. knowlesi* from 641st to 644th day and on 655th and 656th days. *P. cynomolgi* present in very scanty numbers on 664th, 665th and 666th days.

Result. A slight but distinct attack of *P. knowlesi* followed superinfection. A marked relapse of *P. cynomolgi* was also observed at a later date.

History of 6th heterologous superinfection. Superinfected on 666th day with strain K_a from Monkey No. 564 (*S. rhesus*) (passage 4). Blood taken towards the end of the acute primary attack which was being treated.

The very scanty *P. cynomolgi* present at time of inoculation continued until 680th day, when mixed infection detected for few days. *P. knowlesi* alone found in small numbers from 685th to 701st day, except on 694th when a few *P. cynomolgi* also seen. Mixed infection present from 702nd to 708th day, after which extremely scanty infection of *P. knowlesi* detectable till 722nd day; this infection was little heavier from 723rd to 727th day; diminished till 733rd day and replaced by scanty mixed infection lasting till 739th day. After finding *P. cynomolgi* only on 740th day, no parasites again detected till 757th day, except for few *P. cynomolgi* on 753rd. A scanty infection of *P. knowlesi* then developed lasting till 763rd day, when replaced by mixed infection for 2 days. A definite relapse of *P. cynomolgi* followed from 765th to 780th day with maximum from 769th to 774th day. Blood examination again negative for 4 days; slight infection with *P. knowlesi* from 785th to 794th day. No parasites seen until 802nd day, from which time either *P. cynomolgi* or *P. knowlesi* in scanty or very scanty numbers seen daily. Blood examinations again showed no parasites from 820th to 881st day, except for few *P. knowlesi* on 837th, on 843rd to 846th and 862nd to 865th day. On 881st day the animal was placed on a course of treatment in connection with another experiment.

Result. Although no very high numerical prevalence of *P. knowlesi* was detected on any one day after superinfection, yet the tolerance which had previously been more marked was diminished, and parasites were found almost daily over a very long period after superinfection. Mixed infections of this parasite and *P. cynomolgi* were frequently detected, and a distinct parasitic relapse of the latter species occurred.

* This animal was inoculated from a monkey with a mixed infection at the 3rd heterologous superinfection.

Summary of results of 5th and 6th heterologous superinfections in Experiment (vi).

A heterologous superinfection with strain K₆ of *P. knowlesi* superimposed upon previous infections with five other strains of this parasite, resulted in a distinct parasitic relapse. As the infection was taken from the original host of this strain (*S. irus*), which had a mixed infection, it caused a mixed infection of *P. knowlesi* and *P. cynomolgi* in the recipient animal. Several parasitic relapses were also observed, due to the latter parasite.

A later heterologous superinfection with strain K₅ was made. Although this produced no definite parasitic relapse, it appeared to increase the persistence of *P. knowlesi* in the peripheral blood. At this time several distinct relapses of *P. cynomolgi* were also observed.

(vii) Monkey No. 9 (*S. rhesus*).

The detailed history of this animal is given by Mulligan and Sinton (1933b). The monkey had one primary infection and five heterologous ones, the last of which proved fatal.

(viii) Monkey No. 39 (*S. rhesus*).

The detailed histories of the primary infection and of four heterologous superinfections with *P. knowlesi* in this monkey, have been given by Mulligan and Sinton (1933b) in their Experiment (viii). Since that time this animal has received two other heterologous superinfections.

History of primary infection and earlier superinfections. This has been given by Mulligan and Sinton (1933b) (pp. 830-831).

History of 4th heterologous superinfection. Superinfected on 384th day with strain K₅. Blood taken from Monkey No. 128 (*S. irus*) (original host)* during the course of a very chronic infection. Slight transient rise in number of parasites with maximum about 393rd day; parasites absent or very few from 396th to 400th day, and not seen again up to 422nd day. A slight parasitic relapse due to *P. knowlesi* then developed, with maximum on 427th day; parasites absent from 431st to 461st day. Scanty parasites from 461st to 467th day, after which none found up to 489th day.

Result. A very slight parasitic attack due to *P. knowlesi*.

History of 5th heterologous superinfection. Superinfected on 489th day with strain K₅. Blood taken from Monkey No. 162 (*S. irus*) (original host) during the course of a very chronic infection.

No parasites detected until 503rd day, when slight parasitic attack of *P. knowlesi* occurred lasting till 513th day, with maximum on 505th and 506th days. Blood examinations negative from 514th to 559th day.

Result. A slight parasitic attack of *P. knowlesi*.

History of 6th heterologous superinfection. Superinfected on 559th day with strain K₅ from Monkey No. 113 (*S. rhesus*) (passage 2); blood taken during a chronic infection.

No parasites detected up to 628th day, except for very few on 585th, 586th and 592nd days.

Result. No appreciable change in infection. This animal appeared to have a high degree of immunity. To test this it was again inoculated with blood from two other monkeys which had received multiple heterologous superinfections with *P. knowlesi*.

* This animal was suffering from a mixed infection with both *P. knowlesi* and *P. cynomolgi*.

History of later multiple superinfection. Inoculated on 628th day with bloods from following two monkeys (a) Monkey No. 29 [vide Experiment (vi)] inoculated previously at different times with *P. cynomolgi* and strains C, K₁, K₂, K₃, K₄, K₅ and K₆ of *P. knowlesi*, and (b) Monkey No. 80 [vide Experiment (ix)] infected previously with *P. cynomolgi* and strains K₃, K₁, C, K₅, K₄ and K₂ of *P. knowlesi*.

No parasites detected at daily examination until 640th day when scanty infection of *P. knowlesi* lasting 646th day developed. Blood examinations negative till 810th day, except for very scanty *P. knowlesi* on 658th and 659th days. Course of treatment started on 810th day in connection with another experiment.

Result. No appreciable change was produced by the simultaneous superinfection of blood from two monkeys which had previously been inoculated with a multiplicity of strains of *P. knowlesi*.

Summary of results of 4th, 5th and 6th heterologous superinfections in Experiment (viii).

This animal had previously been infected with 4 other strains of *P. knowlesi*, before being superinfected with strain K₅, which caused only a very slight parasitic relapse. The 5th heterologous superinfection was made with strain K₆. This caused a slight parasitic relapse, while a sixth superinfection with strain K₃ produced no appreciable change in the infection.

The high degree of immunity acquired by this animal, as the result of 7 infections lasting up to the 628th day, is shown by its reaction to the injection of multiple strains. The simultaneous inoculation of parasitised blood from an animal which had received infections of 7 different strains, and from one which had received 6 different strains failed to break this immunity.

(ix) Monkey No. 80 (*S. rhesus*).

The histories of the primary infection and of three heterologous superinfections (one with *P. cynomolgi*) are detailed in Experiment (ix) of Mulligan and Sinton (1933b). Since that time this animal has had three other heterologous superinfections.

History of primary infection and earlier superinfections. This has been given in Mulligan and Sinton (1933b) (pp. 831-832).

History of 3rd heterologous superinfection.* Superinfected on 305th day with strain K₅. Blood taken from Monkey No. 132 (*S. rhesus*) (passage 2) during a chronic infection. Slight transient increase of parasites (*P. knowlesi*) from 311th to 315th day; very few or no parasites from 317th to 329th day; then marked relapse due to *P. cynomolgi* from 330th to 336th day; with maximum about 333rd day. Slight parasitic relapse of *P. knowlesi* again occurred from 348th to 350th day and a more marked one from 365th to 383rd, with maximum from 368th to 372nd day; parasites absent in intervals. No parasites found from 384th to 401st day, when marked relapse of *P. knowlesi* occurred with maximum from 406th to 409th day. The number of parasites then decreased and extremely few present on 414th day.

Result. Following superinfection a few transient parasitic relapses of *P. knowlesi* were seen, but after 95 days an acute attack occurred. This relapse was such a long time after the superinfection that one does not seem justified in attributing it to the immediate effects of the last inoculation. A relapse of *P. cynomolgi* was also noted.

History of 4th heterologous superinfection. Superinfected on 414th day with strain K₅. Blood taken from Monkey No. 162 (*S. irus*) (original host) during the course of a very chronic infection.

* This was the 4th superinfection as the animal had been superinfected previously with *P. cynomolgi*, as well as two heterologous strains of *P. knowlesi*.

Few *P. knowlesi* seen on day following inoculation, and present again in very scanty numbers from 418th to 430th day. Then increased, causing a slight but distinct relapse from 432nd to 434th day; after this, infection diminished and no parasites found from 438th to 453rd day. Few parasites seen from 453rd to 456th day, and not again till 467th. Few parasites from 467th to 473rd day and none again up to 480th day.

Result. A slight transient attack of *P. knowlesi* without clinical symptoms.

History of 5th heterologous superinfection. Superinfected on 480th day with strain K₁ from Monkey No. 190 (*S. rhesus*) (passage 2) during a chronic infection.

Very few *P. knowlesi* found next day; none again up to 533rd day, except very few on 489th, 490th, 499th and 500th days. Afterwards few or scanty parasites present on most days up to 553rd; then not detected until 562nd, on which day and on 564th extremely few found.

Result. No appreciable change in infection.

History of 6th heterologous superinfection. Superinfected on 564th day with strain K₁ from Monkey No. 540 (*S. rhesus*) (passage 7). Blood taken during a chronic infection.

Very scanty *P. knowlesi* found on 569th day, and present in scanty or very scanty numbers on most days up to 591st. On 592nd a few *P. cynomolgi* noticed. Blood examinations again negative until 606th day, except extremely few *P. knowlesi* on 603rd. Parasites (*P. knowlesi*) again seen in scanty or very scanty numbers almost daily till 627th day and not again till 637th. From this date till 650th day scanty parasites (*P. knowlesi*) found daily, and then usually very scantily till 672nd day. Again present in scanty numbers from 673rd to 681st day, and not seen again, except from 688th to 690th day, until 700th day. *P. knowlesi* seen in very scanty numbers almost daily from 700th to 730th day. A course of treatment was then given in connection with another experiment.

Result. Although no very great increase in the number of parasites was detected on any one day, yet there is no doubt that this superinfection caused a distinct increase in the general intensity of the parasitic infection. This is shown by the observations that, before superinfection parasites had been absent on and off for long periods, while afterwards they were seldom absent from the peripheral blood for any long interval during a period of observation lasting 165 days*.

Summary of results of 4th, 5th and 6th heterologous superinfections in Experiment (ix).

A superinfection of strain K₁ on the top of infections with 4 other strains of *P. knowlesi* and one of *P. cynomolgi*, produced but a transient attack. The next superinfection, with strain K₂, caused no appreciable change in the infection. A sixth superinfection with strain K₄ was followed by a distinct increase in the parasitic prevalence, although it did not cause any definite relapse. This prolonged occurrence of parasites in the peripheral blood seems to be a feature of this strain, and has been noted previously (Mulligan and Sinton, 1933b).

* The after-history of this animal is very interesting. A course of treatment was started on 731st day in an attempt to produce a radical cure. Quinine was given daily for a week, followed immediately by a course of plasmoquine, also lasting for one week. Treatment was then stopped for one week (745th to 752nd day). During the next week atabrin was administered daily. Parasites, which were present on the first and second days of quinine treatment, disappeared then, and were not again seen up to the end of an observation period lasting 2 months after the cessation of all treatment (821st day). This monkey was superinfected on 822nd day with strain K₁. Parasites were detected in scanty numbers on 828th day, and increased so rapidly in numbers that the animal died with blackwater fever on the night of 830th/831st day. This last fatal superinfection was therefore done 341 days after the animal had previously shown itself refractory to this strain, and 2 months after the cessation of all treatment.

(x) Monkey No. 82 (*S. sinicus*).

The history of the primary infection and those of three heterologous superinfections have been detailed by Mulligan and Sinton (1933b) in their Experiment (x). Since that time this animal has been superinfected with three more heterologous strains of *P. knowlesi*.

History of primary infection and earlier superinfections. This has been given by Mulligan and Sinton (1933b) (pp. 832-833).

History of 3rd heterologous superinfection. Superinfected on 296th day with strain K₆. Blood taken from Monkey No. 162 (*S. irus*) (original host), which had a very chronic infection. Scanty parasites detected from 305th to 307th day; marked increase with maximum on 310th to 312th day; later gradual decrease, parasites remaining very few or absent till 320th day; slight rise from 320th to 323rd day, and then absent up to 364th day, except for very few on 335th to 337th day. Very few parasites detected daily from 364th to 369th day; none again found till 381st, when very few appeared for 3 days. Parasites not again detected at daily examinations up to 402nd day.

Result. A definite parasitic relapse without clinical symptoms.

History of 4th heterologous superinfection. Superinfected on 402nd day with strain C. Blood taken from Monkey No. 100 (*S. rhesus*) (passage 5) during a relapse of a chronic infection.

Very few or scanty parasites detected from 404th to 407th day; none again found till 420th, when short relapse occurred lasting till 426th day, with maximum on 423rd and 424th day. No parasites then seen until 472nd day, except for extremely few on 450th, 454th, 455th and 456th days.

Result. A slight, but definite, parasitic relapse without clinical symptoms.

History of 5th heterologous superinfection. Superinfected on 472nd day with strain K₅. Blood taken from Monkey No. 128 (*S. irus*) (original host) during a very chronic infection.

Very few *P. knowlesi* found on day of inoculation and 3 more days; none found again till 513th day, when very few present for 3 days. Negative findings again reported up to 544th day, except very few parasites from 532nd to 537th day.

Result. No appreciable change in the course of infection.

History of 6th heterologous superinfection. Superinfected on 544th day with strain K₃. Blood taken from Monkey No. 171 (*S. rhesus*) (passage 2) during a chronic infection.

No parasites found till 556th day, when slight attack occurred lasting till 563rd day with maximum on 560th. Negative findings again recorded till 570th day, then parasites in very scanty numbers present almost daily till 587th. No parasites found again till time of death from pneumonia on 640th day.

Result. A slight but definite parasitic relapse without clinical symptoms.

Summary of results of 4th, 5th and 6th heterologous superinfections in Experiment (x).

A superinfection of strain C superimposed upon four other infections, produced a slight but distinct relapse. The 5th superinfection was made with strain K₅ and caused no appreciable change in the course of the infection. At the 6th superinfection, strain K₃ produced but a slight parasitic relapse.

DISCUSSION OF THE EFFECTS OF MULTIPLE SUPERINFECTIONS WITH HETEROLOGOUS STRAINS OF *P. KNOWLESI*.

There appears to be no literature dealing with the effects of multiple heterologous superinfections in human malaria. Experimental work has been done mainly with *P. vivax*, and seems to have been confined entirely to the results of heterologous superinfection with a single strain of parasite in each case. The results so far recorded with heterologous superinfections in man, show no marked differences from those obtained by us in simian malaria. There

TABLE

Summary of results of multiple heterologous superinfections

Number of experiment.	Serial number and species of monkey.	Strains in earlier infections.	HETEROLOGOUS SUPERINFECTION. 1			HETEROLOGOUS SUPERINFECTION. 2			HETEROLOGOUS SUPERINFECTION. 3		
			Days after 1st inoculation.	Strain.	Result.	Days after 1st inoculation.	Strain.	Result.	Days after 1st inoculation.	Strain.	Result.
(v)	7 (<i>rhesus</i>)	2C *	379	K ₁	+++ (RT)	427	K ₂	+	494	K ₂ plus Cyn.	—
(vi)	29 (<i>rhesus</i>)	2C *	309	K ₁	+++ (RT)	357	K ₂	±	423	K ₂ plus Cyn.	+
(vii)	9 (<i>rhesus</i>)	C	343	K ₁	++ (RT)	379	K ₂	+	427	K ₂ plus Cyn.	±
(viii)	39 (<i>rhesus</i>)	C	203	K ₂	+++ (RT)	287	K ₁	+	344	K ₁	+
(ix)	80 (<i>rhesus</i>)	K ₂ and Cyn.	207	K ₁	++ (RT)	274	C	—	305	K ₂	±
(x)	82 (<i>sinicus</i>)	K ₂	173	K ₁	++ (SR)	246	K ₁	±	296	K ₁ plus Cyn.	+

* This indicates that the animal had a homologous superinfection

Explanatory notes. (a) Result:—

- indicates no attack or appreciable increase
- ± indicates a slight transient increase in the
- +
- ++ indicates a definite increase of parasites,
- +++ indicates an attack of moderate severity.
- +++ indicates a very severe attack.

(b) SR means spontaneous recovery. RT means

(c) The thick line indicates the limits of the

I.

with *Plasmodium knowlesi*.

HETEROLOGOUS SUPERINFECTION. 4			HETEROLOGOUS SUPERINFECTION. 5			HETEROLOGOUS SUPERINFECTION. 6			REMARKS
Days after 1st inoculation.	Strain.	Result.	Days after 1st inoculation.	Strain.	Result.	Days after 1st inoculation.	Strain.	Result.	
523	K _s	+	633	K _s plus Cyn.	+	738	K _s	±	Under observation till 954th day, when anti- relapse treatment started.
452	K _s	+	562	K _s plus Cyn.	+	666	K _s	±	Under observation till 881st day, when anti- relapse treatment started.
484	K _s	+	523	K _s plus Cyn.	+++ (D)	Details given by Mulligan and Sinton (1933b).
384	K _s	±	489	K _s plus Cyn.	+	559	K _s	—	Superinfected with mul- tiple strains on 628th day from Monkeys Nos. 29 and 80, with- out appreciable effect in observation period up to 810th day when anti-relapse treatment started.
414	K _s plus Cyn.	±	480	K _s	—	564	K _s	±	Under observation up to 730th day when anti-relapse treatment started.
403	C	±	472	K _s	—	544	K _s	±	Parasites found daily up till 587th day, and not again till died from pneumonia on 640th day. At autopsy no parasites found in blood of internal organs.

before the heterologous ones.

in the number of parasites.

number of parasites.

without the production of clinical symptoms.

recovery following treatment. D means death.

experiments recorded by Mulligan and Sinton (1933b).

is little reason to believe, therefore, that, when similar multiple superinfections are tried in human malaria, the findings will be materially different from those observed with monkeys.

If the results of our work be applicable to the human disease, several important points emerge, connected with the general principles of the treatment of malarial infections in different types of communities. These points, and the relationship of our findings to some epidemiological observations, have been discussed later in this paper.

The results summarised in Table I, suggest that when an animal has acquired a tolerance to two or more of our different strains of *P. knowlesi*, a high degree of protection is produced against the acute clinical manifestations of superinfection, as the result of acute attacks followed by chronic infections. This protection holds good not only against the same strains of parasite, but also against some other heterologous ones. It does not, however, appear to give an immunity against superinfection with all other strains of parasite in every case. In any discussion of our results, these two points must be considered.

(A) TOLERANCE TO ACUTE CLINICAL MANIFESTATIONS.

It was pointed out by Mulligan and Sinton (1933a) that 'a chronic or latent infection with one strain of *P. knowlesi* appears to confer some tolerance to the clinical effects of superinfection with a different strain of the same parasite'. 'This is indicated by an increased tendency for the acute initial attack of the superinfection to recover spontaneously, and by a diminished tendency for such infections to relapse at a later date'. From our present results, and those of Mulligan and Sinton (1933b), it seems as if a chronic or latent infection with two or more different strains of *P. knowlesi* may cause the acquisition, in *S. rhesus*, of a considerable degree of tolerance to the clinical effects of superinfection with most of the other strains used in our work.

This tolerance may be dependent upon two factors, or a combination of these,—(a) a general stimulation of the reticulo-endothelial system of the host, and (b) the presence of several different immunological or 'antigenic' elements in each of our so-called 'strains'.

(a) GENERAL STIMULATION OF THE RETICULO-ENDOTHELIAL SYSTEM.

It has been shown by Cannon and Taliaferro (1931) and by Taliaferro (1934) that, if the host survives, a general stimulation of the reticulo-endothelial system occurs in both avian and simian malarial infections. This observation has been confirmed by the study of much pathological material obtained from monkeys infected in these laboratories (Mulligan).

Mulligan and Sinton (1933b) have pointed out, however, that the marked cellular response of this system is not the main factor in the production of tolerance to heterologous superinfections. Even when a great stimulation of the reticulo-endothelial system has been produced by a prolonged chronic infection, or by numerous superinfections, with one strain, the high degree of homologous tolerance developed, is insufficient to prevent an acute attack of the disease, when the same animal is infected with a heterologous strain of *P. knowlesi*.

It must be admitted that the phagocytic properties of the reticulo-endothelial system play a great part in the destruction of parasites in the body. Yet, even

when its activity has been greatly stimulated by previous infection with one strain, it is usually unable to cope with the effects of superinfection with a heterologous strain of the same parasite (*P. knowlesi*). Some more specific factor is needed to make the reticulo-endothelial system properly active against such a superinfection. Only as the result of an infection does this specific factor appear to be developed, and its nature has never been demonstrated, although it is suspected to be related to the opsonins. As has been shown by the results of our superinfections, this substance is very highly specialised. So much so that it is not even specific for the *species* of *Plasmodium*, but only for the different *strains* of a species. It is, therefore, the main factor in the causation of 'strain' tolerance, and any markedly increased phagocytic action of the reticulo-endothelial system against any particular strain appears to be dependent on this factor.

(b) STRAINS COMPOSED OF DIFFERENT IMMUNOLOGICAL ELEMENTS.

The term 'strain' was used originally in our work to denote infections discovered in different specimens of *S. irus*, naturally infected. It was later found that most of these so-called 'strains' were not identical immunologically (Mulligan and Sinton, 1933a).

It was also found that the tolerance produced by a primary infection with one strain of *P. knowlesi*, even when reinforced by several homologous superinfections over a long period, did not confer any marked degree of tolerance against heterologous superinfection. Later work by Mulligan and Sinton (1933b) showed that infections with two different 'strains' of *P. knowlesi* in *S. rhesus*, often produced a considerable degree of tolerance to the clinical effects of superinfection with a third strain. This suggested that each of our so-called 'strains' might possess several different immunological elements, some of which were common to two or more strains. If this be so, it would help to account for the acquisition of some, at least, of the specific tolerance to other strains, which was observed after several heterologous superinfections had been made in the same host.

While it may be that a 'pure-line' strain of one species may possess a combination of several antigenic elements, there is not much evidence either to support or refute this idea. It seems to us much more likely, in view of the frequency of mixed infections of two or more *species* of *Plasmodium* in our original hosts (Sinton and Mulligan, 1933), that these hosts might also have received infections with multiple *strains* of *P. knowlesi*. Under such conditions, it appears unlikely that all the natural infections from which our strains were derived, were caused by one strain of *P. knowlesi* only. It is very probable, therefore, that many of our 'strains' were mixtures in different combinations of several pure strains, each of which may have its own clear-cut immunological characters*.

* It has not been found possible to differentiate our strains by any morphological method. It might, however, be possible to separate them by obtaining infections from a single parasite, or, if one could easily transmit the *P. knowlesi* infection experimentally with mosquitoes, to inject very small numbers of sporozoites. From a clinical study of our strains, we have evidence which suggests that one at least may consist of two distinct elements. One of these elements appears to be more liable to cause death with blackwater fever, while the other more usually gives rise to a very severe anaemia at a later period of the infection.

As was pointed out by Mulligan and Sinton (1933b), the results obtained in these experiments support the view that several of our strains have immunological factors in common, and that a combination of two or more of them will produce a fairly effective clinical tolerance to superinfection with a different strain. It must be remembered, however, that all our animals were obtained from the same dealer, and were said to have come from the same area, so the infections may merely represent mixtures of the strains common in the natural environments of these monkeys*. We have no evidence to prove that the high degree of clinical tolerance observed in our animals, would be equally effective against a strain of *P. knowlesi* obtained from a distant area. The marked differences which have been recorded between human infections with the Holland and the Madagascar strains of *P. vivax*, suggest that even more marked variations between the strains of a single species of *Plasmodium* from widely separated localities, may occur than have been observed between our strains of *P. knowlesi*, which came from a more limited area.

Under such conditions, it is impossible to draw any general conclusion that infections with two or three strains of one species of *Plasmodium*, or even of *P. knowlesi* alone, are sufficient to give a high degree of tolerance to the clinical manifestations of later infections with one or all strains of the same species. Indeed the results of Experiment (vii) are against such a conclusion.

(B) IMMUNITY AGAINST HETEROLOGOUS SUPERINFECTION.

Although, under our experimental conditions, superinfected animals may show a tolerance to the clinical effects of further superinfection with heterologous strains, they are not always immune to superinfection. This is shown by the fact that, in a large number of instances, definite parasitic relapses can be elicited by superinfection with a heterologous strain of *P. knowlesi* and almost never with a homologous one.

The increase of the number of parasites in the peripheral blood, although distinct, usually never reaches the heights seen in primary infections or primary heterologous superinfections. This indicates that there had previously been some powerful stimulation of the parasitocidal mechanism. Under such conditions, the number of parasites seldom passes the threshold necessary to produce appreciable clinical manifestations†.

(C) FAILURE OF THE TOLERANCE TO PROTECT AGAINST DEATH.

In all our experiments, except the final one with Monkey No. 9, infections with two or more of our strains of *P. knowlesi* appeared capable of producing a premunition, which was clinically effective against superinfection with any other one of our strains of this parasite.

* Boyd and Stratman-Thomas (1933a) report the occurrence of as many as three strains of *P. vivax* with different immunological characters, isolated from infections in patients residing within a radius of only 25 miles.

† As we are unable to gauge with any precision, small variations in the clinical manifestations of chronically infected monkeys, it is impossible to say whether the absence of detectable symptoms is due to (i) a tolerance developed to the 'toxins' of the disease, or (ii) merely a tolerance developed because the parasitocidal mechanism has been able to keep the number of parasites at such a low level that no symptoms are observed, or (iii) a combination of these two factors.

Monkey No. 9, behaved, however, in the same manner as other susceptible animals do after receiving a primary infection, or a primary heterologous one. This animal had survived its primary infection with the help of treatment, and had without such aid overcome the effects of heterologous infections with four other strains of *P. knowlesi*, inoculated during a period of nearly 5 months. In spite of this, a further heterologous superinfection given about 40 days later (523rd day after the primary inoculation), led rapidly to a fatal result.

This finding would indicate that multiple heterologous superinfections will not always protect against further heterologous ones. It is necessary, however, to consider why this animal behaved in a manner different from Nos. 7 and 29, which did not develop such a severe and fatal attack, although they received the same superinfections in the same sequence.

In the last two monkeys, the intervals between the 4th and 5th heterologous superinfections were 100 or more days, while in the first animal it was only 39 days. If, however, the protocols of our experiments be examined, it will be seen that in some instances, although an even shorter interval was allowed between heterologous superinfections, no fatal result was observed*. It will be seen, on the other hand, that this superinfection was the only instance in *S. rhesus* where such a short interval elapsed between an infection with strain K_4 and strain K_6 .

Now, as noted previously by Mulligan and Sinton (1933b), strain K_4 is probably the most virulent of the strains studied by us. This has been shown by the difficulty experienced in establishing a chronic infection with it, because of the numerous deaths which took place in relapses after the primary infection. This seems to indicate either a higher virulence of this strain, or a diminished power of the susceptible host, *S. rhesus*, to develop a tolerance to it. Apart from deaths in early relapses, a feature of this strain has been the number of severe relapses which may occur for many weeks after the primary acute attack, if the animal survives†.

Under these circumstances, a probable explanation of the fatal result in monkey No. 9, is that the superinfection with strain K_6 was given at a time when a severe relapse of strain K_4 was imminent, and the combined effects of these two infections were responsible for the death. In animals Nos. 7 and 29, where a longer period was allowed to elapse between the infections, the tolerance to strain K_4 was probably well developed at the time of superinfection, and the risk of a severe relapse was excluded‡.

* Taliaferro and Taliaferro (1934) report the presence of tolerance to heterologous superinfection with *P. brasilianum* in Central American monkeys, as early as two or three weeks after the primary inoculation. It must be noted, however, that this species of *Plasmodium* appears to be less virulent than *P. knowlesi*. It is also possible that the animals infected had some 'natural tolerance' to this parasite.

† This is very different from most of our observations with other strains of *P. knowlesi* infecting *S. rhesus*, where the manifestations dangerous to life appear to be confined almost entirely to the primary attack and to the first relapse (recrudescence of James). James, Nicol and Shute (1932) mention the observations of Koch, Marchiafava and others to the effect that tolerance to an infection with *P. falciparum* is so quickly acquired, that, as a rule, the risk of pernicious symptoms is confined to the primary attack. This is also the general rule with infections of *P. knowlesi*, but not with all strains. It is possible, therefore, that there may be strains of some of the human malaria parasites which behave in a similar fashion.

‡ Vide footnote on p. 323.

The results obtained with animal No. 9, show that multiple heterologous superinfections will not give such an effective degree of tolerance as to prevent a fatal result under all conditions. The occurrence of a heterologous infection upon the top of an acute relapse, is not an improbable occurrence in human malaria in nature, and is possibly fairly frequent among young children in hyperendemic areas.

(D) CONCLUSIONS

(a) It has been found, under the conditions of our experiments, that latent infections, following upon consecutive infections with two or more of our strains of *P. knowlesi*, are sufficient, in most cases, to cause the development of a tolerance against the more severe clinical results of further superinfection with another strain of this parasite.

(b) It would seem, however, that, if a sufficient period for the development of an effective tolerance, be not allowed to elapse between the acquisition of any two infections, the second of these may be followed by serious results in some cases.

(c) There is much evidence to suggest that some of our 'strains' are not made up of a single immunological or antigenic factor, but are composite. This may have occurred as the result of natural infections with several different strains in the original host of the infection (*S. irus*).

(d) The heterologous tolerance found in our experiments after two or more strains have been used, would be explicable on the assumption that most of our strains were mixed, so the combination of two or more of them became sufficient to stimulate tolerance against our other strains.

(e) It is not justifiable, however, to draw from these results the general conclusion that the latent infection following upon heterologous superinfections with two or more strains of *Plasmodium*, will produce sufficient tolerance to protect against additional superinfection with *all* new strains of the same species. Before drawing any such general conclusion, it would be necessary to test the tolerance of our immunised animals against several entirely foreign strains, obtained from areas distant to those from which ours were derived.

A DISCUSSION OF THE RESULTS OF SUPERINFECTION EXPERIMENTS IN RELATION TO EPIDEMIOLOGICAL OBSERVATIONS IN HUMAN MALARIA.

It has been shown (Mulligan and Sinton, 1933a) that, if the susceptible animal, *S. rhesus*, survives the acute attacks which occur at the commencement of an infection with the very virulent parasite, *P. knowlesi*, an active tolerance or premunition to the clinical effects of the disease is quickly acquired. This tolerance seems dependent for its continuation upon the presence of a chronic or latent infection in the host, and it also holds good against superinfections with the *same strain* of parasite. These findings are paralleled by those reported in human malarial infections.

If, however, such tolerant animals be superinfected with a *different strain* or *species* of *Plasmodium*, the premunition is not completely effective. The clinical manifestations of these heterologous superinfections resemble those seen in primary infections in a susceptible animal, although they may be less severe.

This absence of heterologous tolerance has been found to occur with a large number of different strains in experiments with *P. knowlesi* in *S. rhesus*. It has also been reported with the few strains of human malaria parasite which have been studied in this respect (*vide* Mulligan and Sinton, 1933a).

There is, in addition, considerable evidence from the results obtained in these laboratories, and from the reports of other workers with human and avian malaria, that this acquired tolerance is lost comparatively rapidly, when the infection is completely eradicated, either by natural or therapeutic means, or a combination of these. It would seem, therefore, that for the maintenance of this tolerance, the defensive mechanism of the host requires to be stimulated continually by the products of the same *strain* of parasite. This stimulation occurs in nature as the result of the schizogonic cycle of the *Plasmodia* in the chronic or latent infection. If such infection dies out, or is not kept going by reinfection, the tolerance to the strain tends to disappear.

It may be however, that several of the local strains in an area have some immunological factors in common, and so latent infections with two or more strains may help the development of a tolerance against the clinical effects of heterologous superinfections with another strain possessing some similar immunological factors.

This acquired tolerance may vary in the degree of its efficacy from time to time. These variations are shown by relapses, either clinical, parasitic, or both, and seem to be influenced in certain cases by the physical well-being of the host. The relapses appear to be stimulated by such factors as over-exertion and fatigue, excess of heat or cold, poor or deficient diet, physical injuries, infection with other diseases, etc., etc. It is also probable that the inoculation of an excessive dosage of infection of the same strain of parasite, may upset the tolerance and so cause a return of the clinical manifestations. In any such relapses in an immunised subject, it is rare to get clinical symptoms of the severity of those seen in a primary attack due to the same strain of parasite*.

These findings appear to throw considerable light upon factors governing the distribution, nature and intensity of malarial prevalence in different parts of India, and to afford experimental support for many of the theories which have been advanced, mainly upon epidemiological evidence, to explain these conditions.

In discussing these findings in their relationship to the epidemiology of human malaria, it is necessary to consider (a) the malarial conditions in areas of low endemicity, (b) in areas of high endemicity or hyperendemicity, and (c) in immunised or non-immunised individuals exposed to infection with a new strain of *Plasmodium*.

(A) THE TOLERANCE IN A POPULATION LIVING IN AN AREA OF LOW ENDEMICITY.

The occurrence of areas of low malarial endemicity is usually dependent upon factors such as (i) the absence of numerous effective and homophilic

* During the course of experiments with about 100 monkeys with chronic infections, in only about two instances have we observed fatal relapses with heavy parasitic infections, among those in which a sufficient period has been allowed to elapse for the development of tolerance.

insect carriers of the disease, (ii) the absence of numerous gametocyte carriers easily accessible to the susceptible insect host, (iii) the absence or short duration of meteorological and environmental conditions favourable for the complete development of the parasite up to the infective stage in the mosquito, and (iv) the absence of easy access of such infective insects to a susceptible population.

The factors influencing the spread of the disease under such conditions are unfavourable. As a result the number of infections distributed among the population is relatively few, so that the chances of an individual becoming infected during any one malaria season are considerably reduced. The chances that any one individual will acquire infection with two different strains or species of parasite, are even less. Such circumstances usually make the intervals between the acquisition of infections so long that these tend to die out in many individuals, either naturally or as the result of therapy. With the loss of infection, any tolerance acquired probably disappears fairly rapidly in most cases. Under such circumstances, except in certain areas where conditions for transmission are more favourable, malarial infection may be considered rather as an accident than as a matter of usual or frequent occurrence.

Owing to the absence of opportunity for any general acquired immunity, as the result of repeated and continued infection among the population of such areas, malarial disease is almost as common among adults as among children. The severity and duration of the clinical manifestations in children may, however, be more marked than in adults. There is evidence to suggest that the reaction of the latter to primary infections is less marked than that of children, even in the absence of the stimulus of a prior infection*.

It has been found that, even among the populations of non-malarious countries, individuals may differ very considerably in the degree of their reaction to any malarial infection inoculated into them. Some appear to react very severely, others less so, while some seem to have a considerable power of resistance to infection. The last state may be manifest by the difficulty in producing an infection in such individuals, or if an infection occurs, few or no clinical symptoms may be apparent, the number of parasites detected in the blood may remain very scanty, and spontaneous cure usually takes place rapidly.

One may meet in areas of low endemicity, persons who give histories of chronic malarial infection lasting over several years, or in whom the signs and

* Christophers (1924) noted that individuals entering a hyperendemic area after the age of 6 years, were apparently less susceptible to the effects of infection than those under this age. He could not exclude, however, the possibility that the former class of persons had previously acquired some tolerance, as the result of prior infection in another area. There is evidence to suggest that children are more susceptible, or less tolerant, to the effects of infection with various micro-organisms than are adults. This point has been discussed by Kligler and Mer (1933). Apart from any development of greater resistance as a normal occurrence with advancing age, the results of our work suggest that the following factors must also be considered, in the case of individuals passing from one malarious area to another, (i) the hypertrophy and 'tuning-up' of the reticulo-endothelial system, as a result of prior infection, and (ii) the possible occurrence of some immunological factors in common between the strains of the two areas. These, irrespective of the age factor, may help to make it more easy for the host to deal with any heterologous infection that may be acquired by him in his new environment (Mulligan and Sinton, 1933b).

symptoms of such chronic disease are very marked (anæmia, splenomegaly, cachexia, etc.). Although these infections may be due to a strain of parasite with peculiar powers of survival in, or greater resistance to the defensive mechanism of, the human host*, many of such patients apparently represent some of the more susceptible members of the community, or those who, because of some special environmental factor, are particularly exposed to multiple and repeated infection. Such susceptible persons would probably acquire an infection when the number and virulence of the sporozoites injected by the mosquito, would be insufficient to cause an infection with clinical manifestations in an individual of normal susceptibility. They may also possess a defensive mechanism which is incapable of throwing off the effects of an infection, either naturally or when aided by ordinary therapeutic means. The result of one or a combination of these factors, is that the individual remains chronically ill with malaria over very long periods.

Because of the absence of opportunity for acquiring tolerance to one or more strains of *Plasmodium*, an increase in the amount of malarial transmission, or a lowering of the resistance of the population from any cause (economic stress, etc.), may be followed by a great increase in the incidence of malaria among both children and adults. Under certain conditions fulminant outbreaks may occur, accompanied by great mortality. In such populations, that weeding-out of the naturally susceptible element, which probably occurs in hyperendemic areas, has not taken place (*vide infra*).

(B) THE TOLERANCE IN A POPULATION LIVING IN AN AREA OF HIGH ENDEMICITY OR HYPERENDEMICITY.

Here the conditions are very different from those found in areas of low endemicity. The factors favourable for active and multiple transmission of infection are present for many months of, if not for the whole, year.

An infant born into such surroundings usually acquires an acute infection rapidly under natural conditions, from the clinical manifestations of which it recovers or dies. With the development of a latent or chronic infection, following upon clinical recovery, it acquires a tolerance or premunity to the infecting strain of parasite. This tolerance is maintained at a high level, and prevented from dying out, by frequent re-inoculations of the infection from mosquitoes carrying the same strain of *Plasmodium*.

If this were all the story, the surviving children should have developed within a comparatively short period after birth, a relatively effective tolerance to infection with each of the *common species* of *Plasmodium* occurring in their environment. It has been found, however, that there are from the immunological standpoint, many different strains of the various species of these parasites. It has also been shown that infection with one of these strains does not confer a complete tolerance to the effects of superinfection with a different one. Under these conditions, of frequent reinfection and superinfection, with either homologous or heterologous strains of parasite, a child will probably not have

* Mulligan and Sinton (1933b) record that one of their strains of *P. knowlesi* (K.) was much more liable to produce severe relapses over long periods than were the other strains of this parasite studied by them.

developed an effective premunition to the local types of malaria until it has survived infections with most of all of the local strains and species of parasite*.

The time taken for the development of such an effective tolerance would depend upon (i) the number of infections being spread in a given time, (ii) the number of different strains and species of parasite present, (iii) the degree to which such strains have immunological factors in common, (iv) the time taken by the host to develop an effective tolerance in relation to the virulence of each strain of parasite† and (v) the degree of natural susceptibility of the host and his power to develop tolerance.

If one considers the observations of Christophers (1924) and of many other workers, the children who survive in such hyperendemic areas, have not usually developed a high degree of tolerance until they reach the age of about 6 years. This age probably corresponds, under local conditions, to the time when such children have overcome the acute manifestations of infections with most of the local species and strains of parasite. Continued residence and exposure in the same environments, is followed by repeated reinfections with the same strains, and so this premunition is continued into adult life and is probably reinforced.

This appears to be a reasonable explanation, in the light of our present knowledge, of the process whereby marked tolerance to malarial infections is developed by the indigenous population in such areas.

This acquisition of tolerance in early life, and its maintenance by repeated reinfection in later years, appears to be a characteristic of the inhabitants of many hyperendemic areas. Here the indigenous children suffer from malarial disease, which is usually not present among the adults to any great extent.

Many of these hyperendemic areas are, however, populated mainly by races which have lived there from time immemorial. These people appear to have acquired, by some process of evolution, a higher degree of natural tolerance to the effects of malarial infections, or the power of acquiring a considerable degree of tolerance more rapidly, than exists among individuals of immigrant races introduced into such areas‡. It is quite possible that through ages of exposure to intensive, untreated malaria, individuals with a greater susceptibility to the effects of infection with the local strains of parasite, would be more liable to die in childhood and would thus be weeded out. The race would thus be continued by survivors (adults) having a more marked degree of natural tolerance, or a greater power to develop a tolerance rapidly. Such a trait might possibly be transmitted as a dominant Mendelian character, and so, after ages of residence in a highly malarious region, a race would be evolved in whom the majority of the individuals would possess this character of diminished

* Many of these heterologous superinfections will cause acute symptoms in the host, with a marked increase in the number of parasites in the peripheral blood. This increase in parasite prevalence usually gives rise to an increase in gametocyte production. The latter will, under natural conditions, in hyperendemic areas, lead to an increase in the number of infected mosquitoes, and so to an increased spread of infections in the locality. Thus a vicious cycle is set up, whereby both the number of strains and infections are increased, and the dosage of sporozoites per individual is greater.

† See footnote, on p. 323.

‡ It is hoped to deal more fully with the epidemiological and experimental evidence on 'natural immunity' in another article of this series.

susceptibility, or increased power to develop tolerance, at least in so far as the local strains and species of *Plasmodium* are concerned.

An immigrant population entering such a hyperendemic area from a less malarious one, although possessing a considerable degree of tolerance to the local strains of their native habitat, usually suffers severely from malaria. The child and infant mortality is high among the scanty children who are born alive, and large numbers of the adults either succumb to the disease or are debilitated by it (*vide* Sinton, 1935b). Those who survive probably belong to the less susceptible class of individuals. With this high mortality, and in many instances the absence of intermarriage with the indigenous population, fresh immigration usually takes place to prevent the population dying out, or diminishing in numbers very rapidly. The resultant population is therefore composed of individuals having both resistant and susceptible elements among the adults, and the progeny have similar characters. Such populations, although they may have managed to exist, by reason of fresh immigration, during many years in a highly malarious area, seldom develop more than a partial tolerance to the disease. They never reach that higher degree of premunition seen among the indigenous inhabitants of the area, and which appears to be the result of a natural weeding-out of all but the more resistant elements of the population through countless ages.

Under ordinary conditions, when it is fully developed, this premunition among the indigenous population appears to break down comparatively rarely in the individual's normal environment, if a heterologous strain or species of parasite be not introduced. Minor diminutions in its effectiveness may be produced by factors such as those mentioned previously as exciting causes of relapses.

(C) THE TOLERANCE IN IMMUNISED AND NON-IMMUNISED POPULATIONS TRANSPLANTED TO NEW ENVIRONMENTS.

In the case of populations or individuals migrating to new environments, the amount of malarial incidence occurring among them will depend upon (i) the amount of malarial transmission to which they are exposed during their period of residence, (ii) the number of susceptible individuals present and (iii) the average degree of resistance or tolerance of the population to infections acquired locally.

When non-immune or slightly tolerant populations enter highly malarious areas, the severity and amount of malarial infection among them are usually very great. Severe, localised, epidemic conditions are liable to occur among such collections of people ('tropical aggregation of labour'), accompanied by high morbidity and mortality rates*.

Although a 'salted' population from a hyperendemic area, may exhibit a high degree of tolerance in its native habitat, this premunition may break down to some extent when individuals are transplanted to new and strange environments. The clinical attacks of malaria which develop under such conditions may be caused (i) by superinfection with new strains or species of parasite, or (ii) an increased dosage of sporozoites of either homologous or heterologous

* The main factors responsible for these epidemic conditions, have been discussed by Sinton (1933).

strains of parasite, or (iii) a lowered immunity due to local conditions which predispose to relapse or increased severity of infection, or (iv) a combination of these factors*.

The severity of the malaria seen, under these conditions, among a population which had some degree of 'natural immunity' (*vide supra*), would be much less than among a population in which such a characteristic was more poorly developed.

There is also considerable evidence to suggest that a person who has acquired a high degree of tolerance to the strains of parasite in a certain area, may lose this premunition by prolonged absence from it. This is probably due to radical cure, either by natural or therapeutic means, of those infections with the strains responsible for his acquired tolerance. In the absence of repeated reinfection with such strains, the infections would die out, and the acquired tolerance diminish. When such an individual returned to his native place, he may again develop acute manifestations of malaria, as the result of reinfection with those strains of parasite to which he was previously tolerant.

DISCUSSION OF THE RESULTS OF SUPERINFECTION EXPERIMENTS IN RELATION TO THE PRINCIPLES OF TREATMENT†.

While anti-mosquito measures for the control of malaria may be very successful, when applied under suitable conditions and where they are financially possible, these methods, as at present known, have not provided a solution of the vast problem of rural malaria in India. In our present state of knowledge, we cannot hope to eradicate the disease in these areas by any known method, but only to ameliorate the lot of the unfortunate sufferers. Whatever other means may be employed to achieve this end, treatment must form the first step in any campaign‡.

If one could obtain the ideal anti-malarial drug, one solution of the problem of rural malaria would be found. The chief properties thought necessary in such a drug have been summarised by Sinton (1930). From the point of view of the present article, the two main essentials appear to be that it should be easy to use on a large scale, and that it should produce a radical cure of the infection with a course of treatment not lasting more than a few days.

If such a drug were available, and if it were possible to give systematic and thorough courses of treatment to all members of a malarious community, with subsequent treatment of all those who later developed acute attacks, it should be possible to eradicate malaria, temporarily at least, from the area under control. Patients suffering from acute manifestations should be specially catered for, because it is amongst these that the most dangerous gametocyte carriers are found, *i.e.*, among non-immune children and adults.

Unfortunately we have no such drug, so treatment must be directed mainly towards the amelioration of the disease by the cure, either clinical or radical, of individual patients. For this purpose, the main drugs at our disposal are the cinchona alkaloids, and some of the new synthetic drugs, such as plasmoquine and atebirin. It is not proposed to discuss here the relative merits of these

* The main factors responsible for these epidemic conditions, have been discussed by Sinton (1933).

† This subject is also discussed by Sinton (1935a).

different therapeutic agents for various purposes in malarial control and treatment, but to consider the question of clinical and radical cures, irrespective of the type of treatment employed to obtain them.

The Malaria Commission of the League of Nations in its Third General Report (1933) points out that, if malarial infections be allowed to run on to spontaneous recovery, or be given but the minimal amount of treatment needed to preserve the life of the patient, the resultant chronic infection will produce a tolerance which aids greatly the action of any therapeutic agent employed later. As a result of this experimental work, which has been done mainly with mental cases undergoing malaria-therapy, the Commission appears to advocate a widespread application of this principle in ordinary medical practice. While such a procedure may be possible, safe, and advisable under special conditions, such as those in mental, and other well equipped and well staffed hospitals, it does not seem suitable for routine use under the ordinary conditions of tropical practice.

It was suggested by Christophers and by James some years ago that, in view of the natural process of immunisation which occurs among the population in certain hyperendemic areas, it might be inadvisable, in our present state of knowledge, to advocate very drastic measures of treatment which might upset this beneficial condition. This view is also put forward in the Third General Report of the Malaria Commission (1933), which says "it might be most unwise in some malarious countries to interfere too radically with the natural process by which the indigenous inhabitants acquire immunity to the disease".

In our discussion of the relationship between the results of experimental superinfections and observations upon the epidemiology of malaria, it is pointed out how the nature and degree of tolerance to this disease may vary very considerably under different circumstances. Since the ideal drug is not yet available, these findings appear to indicate that the general principles of treatment should be adapted for the present to suit each of these varied conditions.

From this standpoint, certain broad lines of treatment may be suggested to meet the circumstances of

(a) individuals in whom the risk of reinfection with the same strain of parasite, within a short period after radical cure, is relatively slight, either because of natural environmental conditions, or of anti-malarial measures of various descriptions;

(b) individuals residing permanently in areas where they are liable to reinfection and superinfection (either homologous or heterologous) within a short period after the termination of any course of treatment producing a radical cure; and

(c) individuals exposed to the risk of severe and frequent infection over comparatively short periods only.

A. TREATMENT OF INDIVIDUALS EXPOSED TO COMPARATIVELY SLIGHT RISK OF REINFECTION.

If such persons be not liable to reinfection at frequent intervals, there appears to be no object in allowing them to acquire a tolerance by prolonged chronic or latent infection, if they can be cured more rapidly by therapeutic means. The evidence available goes to prove that such a tolerance will only

be effective against the infecting strain of parasite, and will not last at a high level for any long period after the infection is radically cured.

In areas of low endemicity, the intervals between reinfections with the *same* strain of parasite may be so prolonged, that the immunising infection may have died out before a new one occurs, and the tolerance acquired to the strain be lost, or so diminished, as to be ineffective in preventing a renewal of the clinical manifestations of the disease upon reinfection with the same strain. Apart from this, there is every chance that the person may be reinfected on the second occasion with a heterologous strain or species of parasite, against the clinical effects of which his hard-won immunity may have little protective power. Nor will such a tolerance probably be of much value to him if he becomes reinfected in another locality.

Under these conditions, the patient will have suffered to little advantage the long series of recrudescences, recurrences and relapses needed to acquire a tolerance to his original infecting strain. At the same time, he will have been subjected to the tedium, discomfort and expense of numerous illnesses during the acquisition of this, to him, comparatively useless tolerance. The tolerance due to his uncured infection will in addition be liable to break down under certain adverse conditions predisposing to relapse, such as excessive heat or cold, fatigue, etc., etc.

All these illnesses will have interfered with the normal tenor of his life, and his efficiency as a worker will have been lowered by the chronic infection. His trials will have been to no purpose, if the acquired tolerance does not protect him against the clinical manifestations of reinfection at a later date.

Where the chances of reinfection with the same strain are slight, any attempt to lower the incidence of malarial manifestations among troops or organised labour forces, by the use of minimal treatment to encourage the development of an acquired immunity, appears to us to be unsound in most circumstances. Apart from any question of heterologous reinfection or superinfection, the chronically infected individuals will usually be less efficient, and will always be liable to relapse under conditions of extra strain. The latter circumstance is a most serious one with troops, who, because of their duties, may also be liable to acquire heterologous superinfections when moved from place to place.

From the point of view of the public health worker, such chronically infected individuals are undesirable, because, under certain conditions, they may act as reservoirs of the disease from which mosquitoes may acquire infection and spread it.

It appears to us, therefore, that among individuals situated under conditions where reinfection with the same strain of parasite is unlikely to occur, or only after long intervals, the therapeutic aim should be directed towards the production of a *radical* cure of the infection at the earliest possible opportunity, by such medicinal means as are found most suitable and satisfactory for this purpose.

B. TREATMENT OF INDIVIDUALS EXPOSED TO FREQUENT AND CONSTANT RISK OF REINFECTION.

There is much evidence to support the view that when, either by therapeutic or natural means, an individual is radically cured of an infection with one strain

of *Plasmodium*, he rapidly loses much of any acquired tolerance or premunition which he had developed to this strain as the result of his infection. The data available suggest very strongly that, to maintain any effective degree of such tolerance to the clinical effects of an infection with one strain of parasite, it is necessary for the individual to continue to harbour parasites of this strain. This means that his defensive mechanism must be continually stimulated either by the parasites of his original latent infection, or by continued re-inoculation with the same strain.

If these facts be correct, and they appear to be so, the production of a radical cure of any one infection under the circumstances being discussed, would appear to be of little value, or indeed may prove harmful. If the patient be liable to frequent reinfection, homologous or heterologous, within a very short time after his radical cure, there appears to be little object in curing his infection. Such a cure would, theoretically at least, render him more liable to clinical attacks than if he continued to harbour a latent infection of the original strain, if the intervals between reinfections with the same strain were longer than the duration of his tolerance after radical cure. Even if his tolerance had persisted long enough to prevent clinical symptoms after reinfection, he would probably again acquire a chronic or sub-patent infection. In which case he would be no better off than before his radical cure.

As we have not yet found a practical solution of the problem of the prevention of infection among rural populations in such hyperendemic areas (*i.e.*, by the destruction of mosquitoes, or by other means to prevent the transmission of the disease), attempts to produce a permanent cure of infections would appear to be a waste of money, and probably even harmful in some circumstances.

The provision of sufficient treatment to produce a clinical cure of any acute attacks of the disease, would suggest itself as the most suitable measure to adopt, in our present state of knowledge. Such a system of clinical cure among individuals liable to reinfection with multiple strains and at very short intervals, should not interfere radically with the development of their tolerance to the clinical manifestations of infections with the local strains, nor should it interfere seriously with any immunity already acquired or inherent. The effect of such treatment would be that (*i*) the risk of a fatal result would be diminished, (*ii*) the severity and duration of the attack would be cut short, and (*iii*) the period of physical disability would be curtailed*.

C. TREATMENT OF INDIVIDUALS EXPOSED TO FREQUENT INFECTION OVER COMPARATIVELY SHORT PERIODS.

The methods of malaria control advocated for such individuals or communities are usually applicable only under conditions where discipline is possible.

This type of treatment is used mainly when bodies of men are sent for comparatively short periods into very malarious areas—conditions where it is desirable that as few persons as possible should be incapacitated by the clinical manifestations of malaria. Such conditions are especially liable to occur among troops sent into malarious areas. Here it is essential that as many men as possible should be fit to perform their military duties, and also that the lines

* See also Sinton (1935a) for a discussion of this problem.

of communication and the hospitals should not be overcrowded with sick to such an extent as to cause a serious breakdown or retardation of the operations. Similar conditions also come into action where large bodies of men are congregated together temporarily, as in connection with large engineering works in malarious localities (railways, canals, etc.), under conditions in which it is impossible to protect them adequately from infection. In this category may also be placed the case of individuals travelling through, or living in, malarious tracts, where they cannot protect themselves properly against infection.

Under these circumstances, the use of the so-called 'prophylactic' medication is suitable. It must be remembered, however, that such a procedure is not a true 'prophylaxis of infection' but rather a 'prophylaxis of clinical symptoms'. The medication, by keeping the number of parasites at such a low level that the pyrogenic threshold is not passed, also keeps most of the individuals free from clinical symptoms over long periods. Although some of the less virulent infections may be cured by this prolonged early *treatment* of the infection, especially in those who have some resistance to the disease, many will have attacks at varying periods after the prophylactic measures are discontinued (McNabb and Stewart, 1927)*. One of the actions of 'prophylactic treatment' appears to be that the drug employed (usually quinine) keeps the parasites below fever level in most instances, but at the same time they are numerous enough to stimulate some tolerance to the strains injected. The result is that, when this early curative treatment ceases, if all the infections have not been cured radically, some of them will develop clinical symptoms at longer or shorter intervals afterwards. These attacks are usually less severe than those seen in primary infections.

While some members of the community may acquire a greater or lesser degree of tolerance to some of the local strains of parasite, even without having experienced an acute attack, some will develop clinical symptoms, during the course of the prophylactic treatment. The latter are more likely to occur under conditions of stress or when the individual is exposed to a massive dose of infection, more especially if this contains several different strains or species of parasite. If properly carried out, however, the procedure will (i) lower the average amount of inefficiency in the force, (ii) diminish the amount of morbidity and (iii) reduce the severity of the attacks and so the mortality.

There is evidence to suggest that the severity of the clinical symptoms and the duration of the incubation period of an infection, may be influenced by the quantum of sporozoites which the individual receives. These factors are probably influenced also by the number of different strains or species of parasite which he receives within a short period. All of these will depend upon the amount of transmission which is going on in the locality at the time, and the exposure of the individuals to such transmission. It is evident, therefore, that the dosage of the drug used for clinical prophylaxis which may be effective under one set of circumstances, may be less so under conditions of more severe infection. The dosage of the therapeutic agent used and the frequency of its administration should, therefore, be determined by local conditions and facilities.

The Malaria Commission in their Third General Report (1933) states that the correct plan of 'clinical prophylaxis' is to take a daily dose of 0.4 grm

*Such cases should then be dealt with along the lines laid down in the other two categories of treatment mentioned above.

(6 grains) of quinine daily. While this may be possible for the individual, or in well disciplined forces, it may be very difficult to carry out among large, undisciplined, or poorly disciplined, bodies of men. With such communities one has usually to rely upon larger doses given at longer intervals.

D. CONCLUSIONS.

If one takes into consideration our present state of knowledge and the properties of the anti-malarial drugs available, the results of our experiments with simian malaria, combined with observations upon the epidemiology of the human disease, appear to indicate that some general principles may be advanced as guides to the treatment of malaria in India, under different circumstances.

(a) In areas of low endemicity, and under other conditions where the risk of reinfection with the same strain of parasite is only likely to occur at relatively long intervals, a *radical cure* of the infection should be attempted at the earliest opportunity.

(b) Among populations permanently resident in hyperendemic areas, where proper anti-mosquito measures are not feasible and where infections and reinfections are frequent and multiple, treatment which aims at *clinical cure* only is to be recommended.

(c) Where populations or individuals are exposed to frequent infections, during a relatively short sojourn in places where proper measures of protection are not practicable, *clinical prophylaxis* appears to be the method of choice during that period.

SUMMARY.

(1) The end results of six heterologous superinfections with different strains of *P. knowlesi* into individual specimens of *S. rhesus* and *S. sinicus*, are recorded.

These results show that, in many instances, the premunition or tolerance developed, as the result of chronic infections with two or more of our strains, is sufficient to protect against the severe clinical manifestations which might have followed upon later superinfections with some of *our* other strains of this parasite.

There is not sufficient evidence, however, to show that this tolerance would prove effective against entirely foreign strains of the same species of parasite. The reasons for this view are discussed.

(2) The results of these experiments have been discussed in their relationship to the interpretation of some epidemiological observations upon the development of immunity in human malaria, under different conditions of the prevalence of this disease.

(3) Some suggestions have been made as to the general principles governing the treatment of malaria, in the light of present knowledge, and in consideration of the limitations of the anti-malarial drugs at our disposal.

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ABSTRACTS.

MALARIA SURVEY REPORT ON NIJPAT JAINTIAPUR, SYLHET DISTRICT, ASSAM.*

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(MS. 17 pp.)

[8th March, 1935.]

THIS report records the results of a malaria survey carried out in Nijpat Jaintiapur, Assam, between June 1933 and April 1935.

Nijpat Jaintiapur is a village of about 1,800 inhabitants but, according to the authors, it was formerly a thriving town with a population of approximately 20,000 inhabitants. The decline is attributable to the ravages of malaria. Nijpat Jaintiapur is situated among the foot-hills of the Khasia and Jaintia Ranges, and the climate is subject to extremes of cold in winter and heat in summer. The annual rainfall is very heavy, being on the average about 208 inches. Particulars are given regarding the climate, meteorological conditions, population, occupation, water supply, vital statistics, etc.

The spleen and parasite rates were determined for various sections of the population, and the following statement has been compiled from the authors' findings.

* Copy of the original manuscript has been placed in the Library of the Malaria Survey of India, Kasauli. This is available on loan to workers who wish to consult the original. (Editor).

Age (years).	Number examined.	Number with enlarged spleen.	Percentage with enlarged spleen.	Number of blood films examined.	Number with parasites.	Percentage with parasites.
Under 2 ..	44	32	73	37	29	78
2-10 ..	427	314	73	407	219	54
11-16 ..	79	54	68
Adults .	494	249	50

It is concluded that malaria in this area is hyperendemic.

The following species of anopheline mosquitoes were identified. The figures given in parenthesis indicate the numbers of each species which were dissected. The only species of anopheline in which oocysts or sporozoites were found was *A. minimus* (*vide infra*).

<i>A. aconitus</i> (104).	<i>A. maculatus</i> (4).
<i>A. annularis</i> (897).	<i>A. minimus</i> (205).
<i>A. barbirostris</i> (8).	<i>A. philippinensis</i> (257).
<i>A. culicifacies</i> (6).	<i>A. ramsayi</i> (24).
<i>A. hyrcanus</i> var. <i>nigerrimus</i> (147).	<i>A. umbrosus</i> (1).
<i>A. karwari</i> (6).	<i>A. vagus</i> (106).
<i>A. kochi</i> (3).	<i>A. tessellatus</i> (0).
<i>A. leucosphyrus</i> (1).	<i>A. jamesi</i> (0).

No evidence of malarial infection was observed in any species of anopheline dissected, with the exception of *A. minimus*. Out of 205 specimens of *A. minimus* dissected 3 showed oocysts in the gut, and 5 showed sporozoites in the salivary glands.

In Nijpat Jaintiapur the highest incidence of malaria is observed in May, after which there is a gradual decline to October, followed by a slight rise in November and December. The authors note that malaria is less prevalent among the wealthier and better educated classes who can afford mosquito-nets.

The authors conclude that, although *A. minimus* is probably the chief vector in this area, this requires confirmation. It would not be justifiable to recommend extensive anti-larval measures until the vector or vectors have been definitely determined. Even then it will be doubtful whether anti-larval measures will be a practical proposition in Nijpat Jaintiapur owing to the divers character and large number of breeding places, in proportion to the size and importance of the area. It is felt that the best hope of reducing malarial incidence lies in mass treatment of the population with quinine and plasmoquine. The need for education and propaganda is pointed out. Screening of the bungalows occupied by the local Government officials is recommended.

MALARIA SURVEY REPORT OF SYLHET TOWN, SYLHET DISTRICT, ASSAM.*

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A MALARIA survey of Sylhet town, District Sylhet, Assam, was carried out between October 1932 and January 1933. After this preliminary survey further observations were made with a view to determining the malaria-carrying species of anophelines. The work was continued up to the end of 1934. Sylhet is a town of some 20,000 inhabitants and is the headquarters of the district of the same name. It is situated on an undulating plain and is bounded on the north and east by low forest-clad hills, on the south by the Surma River, and on the west by an area mainly under rice cultivation. Mosquito breeding places are numerous and varied. Rainfall is heavy (160 inches) and humidity high. The authors describe the town in some detail, and give particulars of the climate, occupations of the inhabitants, prices of food, water supply, vital statistics, etc.

In the town itself 2,846 children were examined and the spleen rate was found to be 3.6 per cent. Details of the spleen rates determined in individual wards are given, and these indicate that the spleen rate varied from 0.44 per cent to 7.09 per cent.

The following species of anopheline mosquitoes were identified. The figures given in parenthesis indicate the numbers of each species which were dissected. No infected mosquitoes were detected.

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<i>A. barbirostris</i> (6).	<i>A. kochi</i> (3).
<i>A. aconitus</i> (1,998).	<i>A. minimus</i> (49).
<i>A. annularis</i> (2,858).	<i>A. maculatus</i> (0).
<i>A. culicifacies</i> (18).	<i>A. philippinensis</i> (104).
<i>A. hyrcanus</i> var. <i>nigerrimus</i> (249).	<i>A. ramsayi</i> (120).
<i>A. jamesi</i> (50).	<i>A. vagus</i> (160).
<i>A. jeyporiensis</i> (3).	<i>A. subpictus</i> (0).
<i>A. karwari</i> (4).	<i>A. varuna</i> (3).

The authors note the small numbers of *A. minimus* encountered (only 91 specimens out of a total adult catch of 14,507).

The findings from examinations of blood films may be summarized as follows :—

Species of Plasmodium.	Number diagnosed.	Percentage.
<i>P. falciparum</i> ..	271	55·87
<i>P. vivax</i> ..	137	28·14
<i>P. malariae</i> ..	29	5·97
Mixed infections	42	8·77

The seasonal incidence of malaria commences in May, and gradually increases to reach its maximum in July and August, after which it gradually declines. The seasonal prevalence of the various species of anophelines is indicated.

The authors conclude that in view of the apparently low incidence of malaria in Sylhet, and the absence of any definite information as to the specific vector or vectors, the institution of any large scale anti-larval measures is at present contra-indicated. They emphasize the need for further investigation, especially with regard to the determination of the dangerous anophelines by dissection. They are of opinion that systematic treatment of all patients and carriers with quinine and plasmoquine is the best course to follow, until further investigations have been carried out.

Addendum.—Since the submission of this report, the authors inform us that, as the result of further work up to May 1935, the total mosquito catch has been increased to 20,818 out of which only 94 specimens of *A. minimus* were recorded. They have also increased the number of dissections of this species from 49 to 52, and found one specimen infected with sporozoites in the glands.

H. W. M.

SOME NOTES ON MALARIA IN BALUCHISTAN.

BY

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[11th July, 1935.]

IN May 1935, the author was detailed to investigate and report upon the prevalence of malaria in Quetta and Fort Sandeman, Baluchistan, and to indicate what steps should be taken in these stations towards the eradication or amelioration of the disease.

A period of 15 days was spent in Baluchistan in May 1935 to make a preliminary reconnaissance in the non-malaria season. It was soon realized that the malaria problem in Quetta was an important one, and that comparatively little was known regarding the epidemiological factors. As the result of this preliminary investigation several interesting and important facts were brought to light, but sufficient information was not obtained to justify the recommendation of any control measures, other than those which would not involve any appreciable capital outlay.

Immediately following the conclusion of this investigation, Quetta was the site of a violent earthquake which practically wiped out the city and civil station. In the hope that it might be of value in considering any programme of reconstruction, a report was prepared indicating, as far as is at present possible, the most salient features of the malaria problem in Quetta before the occurrence of the earthquake. A second report was also prepared as a guide for the prevention and control of malaria during the ensuing malaria season, under the conditions likely to prevail following the earthquake. Abstracts of these two reports are given below.

During this tour in Baluchistan, a brief visit was made to Fort Sandeman in the Zhob Valley, as the result of which a short report was prepared. An abstract of this report is also given below.

(1) REPORT ON A BRIEF MALARIA SURVEY OF QUETTA, BALUCHISTAN, CARRIED OUT IN MAY 1935.*

(MS. 42 pp.)

The military station at Quetta is one of the largest in the British Empire. The garrison consists of nearly 10,000 troops. The adjoining civil station is the seat of the Baluchistan Administration, a large police force, and an important railway centre. The combined population of the city and civil station, exclusive of the military area, is approximately 60,000 during the summer months, but this is reduced by some 20,000 in the winter. Quetta is situated on a plain, 5,000 feet above sea level, and is surrounded on all sides by high mountains. The climate is subject to extreme variations, being hot in summer and intensely cold in winter, the average annual rainfall being in the neighbourhood of 10 inches.

Past history suggests that Quetta has been a very malarious place for many years, and at the present time there is no indication of a permanent reduction in its incidence. Malaria is a serious menace to the community as a whole. The high incidence among the troops is not only the cause of loss of military efficiency in peace time, but a serious danger in the event of war or mobilization, in the malaria season. In the civil area malaria is not only a source of suffering and distress to the general public, but a cause of serious economic loss to the Baluchistan Administration, and the railway authorities.

A spleen census of over 600 children was taken in different localities and revealed spleen rates varying from 10 per cent to 78 per cent. It was decided from these findings that the distribution of malaria in the area was definitely 'patchy', and that the degree of malarial incidence may vary greatly in places at no great distance apart. The reason for this is probably to be found in the close proximity of dangerous breeding places to those localities in which malaria is most prevalent.

The factors influencing the transmission of malaria in Quetta have been discussed at some length. From the evidence available it appears that the most prevalent anopheline mosquitoes in Quetta are species which are known to be dangerous malaria carriers in other localities (*A. culicifacies*, *A. stephensi* and *A. superpictus*). Anopheline breeding places in Quetta include natural springs and seepages, river beds, irrigation channels and leaks and overflows from them, 'karczes'† at least in those parts of their courses where they resemble ordinary open earth irrigation channels, artesian and other wells, and tanks, cisterns, etc.

One is struck by the paucity of definite information with regard to the seasonal prevalence of the various species of anophelines, and the absence of any information with regard to the vector or vectors, and their habits and favourite breeding places in the area. These lacunæ make it essential that a detailed malaria survey should be undertaken before it would be justifiable to

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† A 'kareze' is an irrigation channel which in the upper part of its course is mainly underground, but is exposed to the light at intervals by deep shafts resembling well shafts. By this means water is carried for long distances for irrigation and other purposes. 'Kareze' water ultimately comes to the surface and is led away in open earth channels.

recommend any control measures of an extensive and costly nature. In the absence of such information certain tentative suggestions have been made for the temporary amelioration of malaria. These include the strict control of water admitted to Quetta for irrigation purposes, and the careful supervision of irrigation channels to prevent the occurrence of overflows and leaks. Temporary measures have also been advocated for other more permanent breeding places of different kinds. It is considered that these measures might be supplemented, for the present, by mass treatment at certain seasons of the year.

(2) SUGGESTIONS FOR THE PREVENTION AND CONTROL OF MALARIA IN QUETTA UNDER CONDITIONS WHICH ARE LIKELY TO PREVAIL FOLLOWING THE EARTHQUAKE.*

(MS. 8 pp.)

It is well known that malaria is a disease which is liable to particularly severe outbreaks under conditions of poverty, privation, exposure and overcrowding, if other factors be favourable for its spread. Such conditions, usually described as 'tropical aggregation of labour' are likely to occur in an area devastated by a severe earthquake on the eve of the malaria season. The author has attempted to visualize the conditions prevailing in Quetta after the earthquake from the press and other reports, and to apply the results of his observations made just prior to the earthquake (*vide supra*) towards the prevention of a severe outbreak of malaria among the survivors.

The first point considered is the selection of a healthy site for the refugee camp. He points out that the site utilized for immediate relief work is probably one of the most malarious areas in the Quetta vicinity, and suggests that, if practicable, a potentially less dangerous site should be chosen.†

In the absence of any definite information with regard to the favourite breeding places of the dangerous anophelines of the locality, the only course open is to control mosquito breeding in all water within a half mile radius of the camp. For this purpose he advocates the widespread use of Paris green. Irrigation water should be diverted from the vicinity of the camp wherever possible.

Recognizing the liability for mosquitoes in large camps to aggregate in enormous numbers under the roofs of tents in the early morning, the author recommends measures directed towards their destruction at this time. There is here an excellent opportunity for destroying large numbers of mosquitoes which have fed on the inhabitants and are therefore liable to develop infectivity at a later date. The simplest and most effective method of destroying such mosquitoes is by the use of a suitable insecticidal spray. Many efficient insecticides are on the market, but probably the cheapest and most effective is a kerosene oil mixture containing 'Pyrocyde 20'.‡

* Copy of the original manuscript has been placed in the Library of the Malaria Survey of India, Kasauli. This is available on loan to workers who wish to consult the original. (Editor).

† It is understood that this recommendation has already been acted upon.

‡ *Vide* paper by Sinton and Wats on pp. 275-306 of this number.

Under conditions such as are likely to prevail at Quetta during the coming malaria season, much good can, in the author's opinion, be done by the distribution of quinine or cinchona febrifuge. The action of these drugs is beneficial, not in preventing infection, but in cutting short attacks of malaria, in lowering mortality, and in reducing the reservoir of infection. He emphasizes the necessity of making these drugs *easily* available to the afflicted population, or preferably of ensuring their distribution by voluntary workers. The broadcast use of drugs other than some form of the cinchona alkaloids is *definitely to be avoided*.

The report concludes with some considerations regarding a possible programme for the reconstruction of Quetta. It is pointed out that every effort should be made to rebuild on healthy sites. Important places previously located on very malarious sites and now destroyed were the Railway quarters, the Police Lines and the Royal Air Force Lines. It is considered that in a place like Quetta in which the distribution of malaria is evidently 'patchy' it might be possible to find healthy sites at no great distance from the previous ones. Owing to the nature of the breeding grounds, their permanent eradication from the old sites might be a very expensive undertaking.

A strong plea is made for a proper malaria survey before any reconstruction work is commenced. This applies equally to a plan for rebuilding on or near the previous site, or on a site remote from the previous one.

(3) NOTE ON MALARIA AT FORT SANDEMAN, BALUCHISTAN.*

(MS. 9 pp.)

The important military station of Fort Sandeman is situated in the broad valley of the Zhob River, at an elevation of about 4,000 feet above sea level. At the present time Fort Sandeman is admitted to be one of the most malarious military stations in India.

As a result of a brief visit the author considered that, while the high incidence of malaria in this station may be attributable to a variety of causes which require detailed investigation, there are certain obviously dangerous conditions which should receive immediate attention. The impression gained was that much of the malaria could be accounted for by uncontrolled irrigation in close proximity to barracks. Not only is the supply of irrigation water for certain parts of the station in excess of actual requirements, but no care is exercised in the proper maintenance of many of the irrigation channels. Overgrown irrigation channels, over-irrigated lands, and seepages and leaks from faulty channels are particularly abundant in the vicinity of the Political Agent's residence, and within a short distance from barracks. These places form ideal breeding places for dangerous malaria-carrying mosquitoes.

The solution of this part of the malaria problem in Fort Sandeman lies in the rigid control of the amount and method of supply of irrigation water. The author recommends that the amount of irrigation water admitted to the station should be reduced to the barest minimum commensurate with actual requirements. This should be regulated at the headworks of the irrigation

* Copy of the original manuscript has been placed in the Library of the Malaria Survey of India, Kasauli. This is available on loan to workers who wish to consult the original. (Editor).

system. Owing to the configuration of the country which favours leaks and seepages, and the practice of removing water by digging holes in the banks of the irrigation channels, the present open earth channels are considered unsafe in the immediate vicinity of the station. These defects can best be rectified by the construction of a masonry channel for the 'main' supply. The provision of a channel of this type appears to offer the only possibility of preventing leaks, seepages, and the unauthorized removal of water. A very important part of a scheme of this sort is the provision of proper 'gates' for the distribution of water to the subsidiary irrigation channels. These must be water-tight, and perhaps the best type for this purpose would be pipes with screw-down valves which could be kept locked. A properly devised programme for irrigation in rotation should enable all subsidiary open earth channels to be dried off for several consecutive days in each week.

The existing domestic water supply for Fort Sandeman is brought into the station in pipes for a distance of about 10 miles, and is therefore vulnerable from a military point of view. Attempts are now being made to provide a reserve water supply from wells within the military area. The author points out that the sinking of wells is likely to be followed by increased water distribution in the station, which is almost certain to result in the formation of additional dangerous mosquito breeding places. He recommends that the wells sunk should be limited to the smallest possible number needed for emergencies. They should be reserved for such use, and their working under ordinary conditions should be reduced to the minimal amount which would keep the wells in good condition.

Other potential dangers are also considered, and the author concludes with the remarks that, even if these recommendations are properly carried out, there will still remain other contributory factors to be met, studied and tackled. It may be that some of these problems may prove to be major ones.

J. A. S.

STUDIES ON MALARIA IN VILLAGES IN WESTERN BENGAL.

BY

HARRY G. TIMBRES, M.D. (Johns Hopkins), D.T.M. & H. (Eng.).

[28th April, 1935.]

I. *A. PHILIPPINENSIS*, *A. ANNULARIS** AND *A. PALLIDUS* AS CARRIERS OF MALARIA.

THE Malaria Survey, in which these studies were made, covered seven villages lying in an area of $9\frac{1}{2}$ square miles in the jurisdiction of the Ruppur Union Board, Bolpur Thana, District of Birbhum, Bengal. The nearest railway station is Bolpur, 4 miles east of the centre of the surveyed area, on the East Indian Railway Loop Line. Suri, the District Town, is 25 miles to the north. The survey was continued for 89 weeks, beginning on 13th July, 1932, and ending on 31st March, 1934. It was made possible through the help of the Institute of Rural Reconstruction, which is the Village Work Department of the Visva-Bharati, founded by Dr. Rabindranath Tagore, and the American Friends Service Committee of Philadelphia, U. S. A., who lent the services of the author to direct the survey.

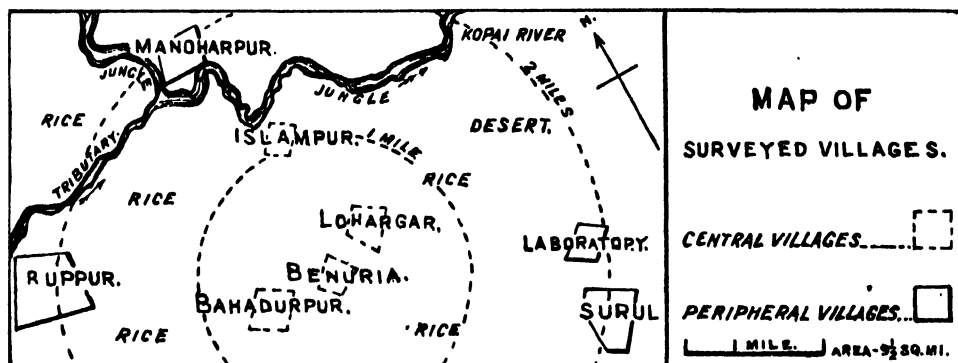
A. PHYSICAL CHARACTERS OF THE REGION.

The country is flat, its general slope being from the north-west to the south-east. Six miles from the surveyed area, the Ajoy River, a tributary of the Ganges, flows eastwards and divides Birbhum District from Burdwan District on the south. A small, permanent stream, the Kopai, also flows through two of the surveyed villages (*see Map*).

* The author in his MS. uses the name *A. fuliginosus* Giles for this species, but more recent work has shown it to be synonymous with *A. annularis* Van der Wulp. (*Editor*.)

Much of the soil is laterite and is devoted largely to the cultivation of rice. Irrigation channels are shallow and come from small storage lakes (or tanks) most of which contain aquatic vegetation. In all of the villages the total surface area of these tanks exceeds the area occupied by the houses, cowsheds, and trees. Wells are scarce.

MAP.



There are no swamps in this region. Rain-water drains rapidly from the surface of the ground. The level of ground water, determined in a well at the centre of the surveyed area, was 35 feet from the surface level at the end of March 1933, and 14 feet in the middle of August 1933.

B. CLIMATE.

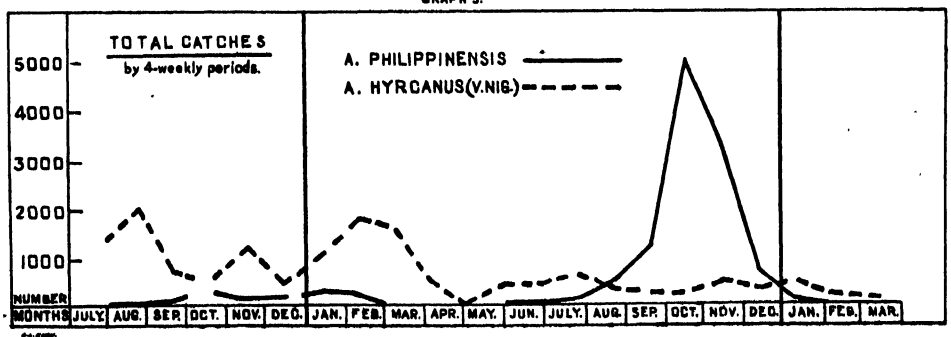
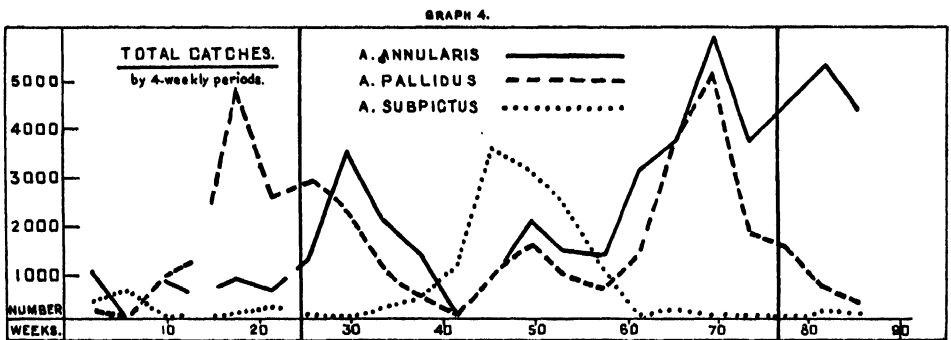
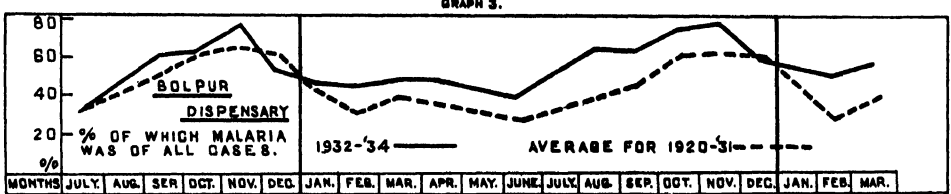
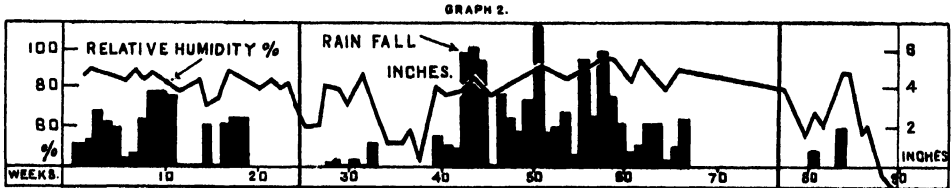
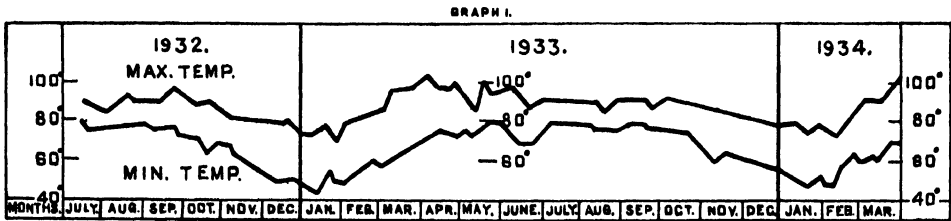
The average annual rainfall for 1929-1931, recorded at the Meteorological Station of the Institute of Rural Reconstruction, which is situated on the periphery of the surveyed area, was 56.4 inches. During the period of the survey the total rainfall was 112.2 inches. The rainfall during 1933, when the monsoon started about 2 months earlier than usual, was 74.8 inches. The conditions of temperature, relative humidity, and rainfall during the period of the survey are shown by weeks in Graphs 1 and 2.

C. HISTORY AND PREVALENCE OF MALARIA IN THE DISTRICT.

On the Malaria Map of India (Christophers and Sinton, 1926) Birbhum District is shown to be on the edge of the hyperendemic zone of Northern and Central Bengal. Prior to 1872, both Birbhum and Burdwan Districts were considered to be healthy. From 1872 to 1874 there was in the summer and autumn of each year an epidemic of fever, which, according to the Statistical Account of Bengal (1886, Vol. IV), caused fatalities of up to 28 per cent in the worst affected villages. This account reports that in 1865, fevers constituted 8.3 per cent of all cases of illness treated in the Birbhum Dispensaries, and in 1881, they constituted 61.8 per cent. With few exceptional years, the fever percentage in these dispensaries has been close to this level ever since.

Graph 3 shows the monthly percentage of all diseases treated which were found by cases diagnosed clinically as malaria. These observations were made between 1920 and 1931 at the Bolpur District Board Dispensary, four miles

GRAPHS 1 TO 5.



from the centre of the surveyed area. From this graph it will be seen that there is a major autumnal rise of malaria reaching a peak in mid-November, and a minor spring rise, with its peak in mid-March. In this area there is a high level of fever prevalence, similar to that in other zones in which malaria is hyperendemic. From Graph 3 it can also be seen that the malaria experience of the Bolpur Dispensary, during the period of survey, did not depart materially from the normal.

The surveyed villages, as do most of the villages in this region, show ample evidence in their large numbers of deserted and ruined dwellings, of having been considerably more populous than they are at present. The Statistical Account of Bengal (1886) reports the populations of two of the surveyed villages, Islampur and Manoharpur, as 885 and 835, respectively, in 1870. A census, taken by us in March 1933, showed the population of these two villages to be 183 and 324, respectively. This reduction has not, however, been typical of Birbhum District as a whole, the population in which has increased from 696,000 in 1872 to over 900,000 in 1931. There was a 13 per cent increase between 1921 and 1931.

D. THE SURVEYED VILLAGES.

The names and populations of the surveyed villages, as determined by our census in 1933, were :

<i>Name of village</i>		<i>Population</i>		<i>Classified as</i>
(1)	Bahadurpur	..	326	Central Villages.
(2)	Benuria	..	321	
(3)	Lohargar	..	590	
(4)	Islampur	..	183	
(5)	Ruppur	..	421	Peripheral Villages.
(6)	Manoharpur	..	324	
(7)	Surul	..	417	
Total		..	2,582	

With the exception of Surul village the entire population of all other villages was included in the survey. In Surul only 417 persons, or 79 families, out of a total population of about 1,200, were included.

In regard to the social and economic condition of the population, the nature of the soil, the type of agriculture, and in other general respects, these villages may be regarded as typical of a large part of the southern half of Birbhum District.

Because of some differences in the plan of the survey in different groups of villages, it will be convenient to refer to the first four named above as 'Central Villages', and to the last three as 'Peripheral Villages'.

E. STATUS OF MALARIA IN THESE VILLAGES.

The results of spleen and parasite surveys performed in these villages are shown in Table I. For comparison, the results of similar surveys performed in neighbouring villages are shown in Table II. The spleen rate was taken as

TABLE I.

Spleen rates, average enlarged spleen, parasite rates and gametocyte rates of Central and Peripheral Villages from April 1932 to March 1934.

Date.			Central Villages.	Peripheral Villages.
1932	April and May	Spleen rate	88.5 per cent	60.5 per cent
		Average enlarged spleen (apex-umbilicus \times apex-midline).	7.5 cm. \times 5.3 cm.	8.2 cm. \times 6.6 cm.
	October ..	Spleen rate	76.0 per cent	67.0 per cent
		Average enlarged spleen	8.1 cm. \times 6.7 cm.	7.4 cm. \times 5.7 cm.
		Parasite rate	37.6 per cent	
	Gametocyte rate ..	13.5 "		
1933	April and May	Spleen rate	66.8 per cent	48.0 per cent
		Average enlarged spleen	7.7 cm. \times 6.6 cm.	7.9 cm. \times 6.5 cm.
		Parasite rate	31.2 per cent	42.4 per cent
		Gametocyte rate ..	13.5 "	21.7 "
	October ..	Spleen rate	81.5 per cent	68.5 per cent
		Average enlarged spleen	7.2 cm. \times 6.1 cm.	7.6 cm. \times 5.8 cm.
		Parasite rate	27.2 per cent	41.2 per cent
		Gametocyte rate ..	10.0 "	18.9 "
December ..	Parasite rate	29.2 per cent	51.1 per cent	
	Gametocyte rate ..	12.7 "	31.7 "	
1934	March ..	Spleen rate	87.5 per cent	59.2 per cent
		Average enlarged spleen	8.6 cm. \times 7.6 cm.	7.3 cm. \times 6.0 cm.
		Parasite rate	17.3 per cent	33.3 per cent
		Gametocyte rate ..	5.0 "	19.1 "

Plasmoquine distributed to all children, 0-14 years, and fever cases in Central Villages from July 1933 to February 1934.

TABLE II.

Spleen rates and average enlarged spleen of certain neighbouring villages.

Village.	Distance from centre of surveyed area.	Date.	Spleen rate (per cent).	Average enlarged spleen (apex-umbilicus \times apex-midline).
Bandgora ..	3½ miles	April 1932	20.0	13.6 cm. \times 11.6 cm.
Mehidipur ..	1½ "	" 1933	13.0	
		" 1932	22.1	9.9 cm. \times 8.2 cm.
Kendanga ..	3 "	" 1933	29.8	
		" 1932	53.0	8.8 cm. \times 8.0 cm.
Raipur ..	6 "	" 1932	85.0	6.2 cm. \times 4.2 cm.
Ballavpur ..	2½ "	" 1932	94.1	6.3 cm. \times 5.4 cm.
		" 1933	66.6	6.7 cm. \times 5.8 cm.

the percentage of all children* showing enlarged spleen. The average enlarged spleen was measured in centimetres, apex-umbilicus, apex-midline, standardized to nipple-umbilicus measurement by the method of Christophers and Khazan Chand (1924). The parasite rate was taken as the percentage of all children* from whom blood slides were taken, showing parasites. The gametocyte rate was taken as the percentage of all children, from whom blood slides were taken, showing gametocytes. In all villages named, not less than 60 per cent of all the children, as found at house-to-house visitations throughout each village, were examined for enlarged spleen. In the Central Villages not less than 70 per cent of the children were examined in this way. Blood was taken from 90 per cent or over of the children examined for spleen. Thick smears were used, stained with Giemsa's stain.

Of the Central Villages, Bahadurpur, Benuria and Lohargar consistently showed very high spleen rates, always above 72 per cent in the spring, and above 83 per cent in the autumn. Lohargar had a spleen rate of 97 per cent in October 1933. These villages showed correspondingly large average spleens. Islampur, on the other hand, had a spleen rate of about 40 per cent up to May 1933. In October 1933, it rose to 69.7 per cent, and in March 1934, to 71.1 per cent. The average enlarged spleen of this village, however, remained fairly constant being about 8.8 cm. \times 7.7 cm.

In the Peripheral Villages, the spleen rates and the sizes of the average enlarged spleen remained fairly constant around the average for this group.

From Tables I and II and the information given in the last two paragraphs, it may be seen: (1) that for the area as a whole the spleen rate was high and the average spleen was large; (2) that in individual villages not far apart there was considerable variability in the spleen rate, this being as low as 13 per cent in Bandgora in April 1933 (Table II), when it was 66.8 per cent in the Central Villages (Table I); (3) that wide variations in spleen rates in individual villages from season to season and from year to year, as were observed in Islampur, were rather exceptional, indicating that the 'patchy' distribution of malaria was rather constant; (4) that as compared with the Peripheral Villages, the Central Villages showed a higher spleen rate and a larger average spleen, but a lower parasite and gametocyte rate.

The distribution of Plasmodia in the first and second halves of the year, both years taken together, is shown in Table III. The numbers in this table are made up of all the blood slides taken during the various spleen surveys, *plus* the slides received with Daily Fever Reports from July 1933 to February 1934, in the Central and Peripheral Villages. It would seem from this table that benign tertian malaria played the chief rôle in the autumn epidemics of 1932 and 1933, and that malignant tertian was of secondary and quartan was of even less importance. In the spring of 1933 and 1934, however, quartan superseded malignant tertian and became next in prevalence to benign tertian in the surveyed villages.

THE PREVALENCE OF KALA-AZAR.

In a dispensary conducted in Benuria chiefly for the benefit of the Central Villages, 121 Aldehyde tests were done during the period of the survey. Of

* In this survey the term 'children' is used to denote those from the age 2 to 14 years inclusive.

TABLE III.

Distribution of Plasmodia in autumn and spring epidemics.

Time.	<i>Plasmodium.</i>	Number of infections observed.	Per cent of total infections.
Summer and Autumn (August to December, 1932 and 1933).	<i>vivax</i>	507	58.2
	<i>falciparum</i>	289	33.2
	<i>malariae</i>	75	8.6
	TOTAL ..	871	100.0
Winter and Spring (January to May, 1933 and 1934).	<i>vivax</i>	129	47.0
	<i>falciparum</i>	60	21.8
	<i>malariae</i>	86	31.2
	TOTAL ..	275	100.0

these, 98 were strongly positive. Their distribution in the Central Villages was: Bahadurpur, 8, Benuria, 6, Islampur, 2, and Lohargar, 82. A series of 450 Chopra Flocculation tests done in Lohargar in March 1933, on persons of all ages taken at random without regard for the condition of the spleen, showed 68 (15.2 per cent) strongly positive. Of these 68 persons, 46 (67.6 per cent) had palpable spleens, and 16 (23.5 per cent) had spleens extending to the umbilicus. Taking palpable spleen *plus* strongly positive Chopra Flocculation test as good presumptive evidence of kala-azar, it would seem, therefore, that at least 10 per cent of the inhabitants of Lohargar were infected with this disease. This fact should be taken into account in considering the high spleen rate of that village. Judging from the Aldehyde tests done in the Benuria dispensary, it would seem that kala-azar did not play so important a rôle in raising the spleen rates of the other three of the Central Villages. We have no evidence, one way or the other, as to the prevalence of this disease in the Peripheral Villages, but we feel safe in assuming that it did exist.

PLAN OF THE SURVEY.

As it relates to the capture and dissection of adult anopheline mosquitoes, the survey may be considered in two periods: (1) from 13th July, 1932 to 31st May, 1933; and (2) from 1st June, 1933 to 31st March, 1934. During the first part, most of the catches were made in the Central Villages, in houses and cowsheds, during the day only. There were relatively few hours spent in night catching. During the second period emphasis was placed upon night catches in houses, but day catches in both houses and cowsheds were also continued. The Peripheral Villages were included in the catching area at the beginning of the second period.

The houses and cowsheds used as catching stations were distributed at random points in the villages. They were not always the same, but over a period of a week of catching, the houses and cowsheds in which catching took place in any village were a good random sample of the village as a whole.

There were never less than four collectors at work at any time, except from 3rd to 16th October, and 20th to 29th December, 1932, when no catching was done. Most of the time during the first period there were six, and, during the second period, eight collectors at work. They worked $5\frac{1}{2}$ days per week, the day men working 6 hours and the night men 4 hours in twenty-four. House catches including those at night were always made in rooms used for sleeping.

Only salivary glands were dissected. During September 1932, about 3,000 mosquitoes were dissected by Sergeant's method. It was found, however, that this method was no more rapid than dissecting the salivary glands, and hence the latter procedure was resumed and adhered to for the rest of the survey. The average number of dissections per working day of the period of the survey was 208.

The synoptic table of Christophers *et al.* (1931) was used for identification of the anopheline mosquitoes. Male mosquitoes were not counted.

Catches of larvæ were made by two men working regularly in the Central Villages from 14th June to 27th December, 1932. These men made rounds of all the collections of water in the Central Villages, so that each breeding place was examined for larvæ at least once every two weeks. An average of five dips were made at each visit.

Daily Fever Reports were obtained from all the villages from 11th July, 1933 to 21st February, 1934. Men trained in taking thick blood smears visited every family in each village daily, and took a blood smear from every person sick with fever within the past 24 hours who had not previously reported within the past week. Patients having fever, but not permitting blood examination, are not included in Table VII. This practice excluded from the fever record about 5 per cent of the reported cases.

ANOPHELIISM OF THE SURVEYED AREA.

The species of anophelines found in the area, the numbers caught and dissected, and the months of the greatest prevalence of each species, are shown in Tables IV, V and VI. A total of 154,260 specimens were caught and identified, of which 98,913 were dissected. Fifteen different species of anophelines were encountered. From the larval collections 7,154 specimens were identified either as bred-out adults or as 4th stage larvæ. Three of the least prevalent species captured as adults were not found as larvæ (Table VIII).

A. philippinensis, *A. annularis* and *A. pallidus* as carriers of malaria.

These were the only species found at any time of the survey to have infection in their salivary glands. The records of the catch, dissection, infection and sporozoite rate of infected specimens of these three species are given for 4-weekly periods in Table IV.

The records of the fever and malaria cases, as well as the sporozoite rate of *A. philippinensis*, in 4-weekly periods, from 11th July, 1933 to 21st February, 1934, are shown in Table VII. The record of the Central Villages is omitted from this table since a considerable amount of anti-gametocyte

TABLE IV.

Catches, dissections of salivary glands, and sporozoite rates of A. philippinensis, A. annularis and A. pallidus by 4-weekly periods from 13th July, 1932 to 22nd March, 1934 and for 8 days 23rd to 31st March, 1934, from all the surveyed villages.

Date.	<i>A. philippinensis.</i>				<i>A. annularis.</i>				<i>A. pallidus.</i>				TOTAL OF THREE SPECIES.			
	* Cat.	Diss.	Inf.	Rate per cent.	Cat.	Diss.	Inf.	Rate per cent.	Cat.	Diss.	Inf.	Rate per cent.	Cat.	Diss.	Inf.	Rate per cent.
1932																
13.vii-8.viii	10	10	1,140	1,009	140	96	1,280	1,115
9.viii-6.ix	18	18	198	87	110	78	326	183
7.ix-3.x	100	100	2	2.0	841	604	896	624	2	0.3	1,837	1,328	4	0.3
4.x-1.xi	322	322	1	0.3	493	264	1,308	870	1	0.1	2,123	1,456	2	0.14
2.xi-29.xi	182	182	992	652	4,821	2,636	2	0.08	5,995	3,470	2	0.06
30.xi-27.xii	200	200	635	533	2,608	2,277	3,443	3,010
1933																
-23.i	315	315	1,389	1,191	2,895	2,214	1	0.05	4,599	3,720	1	0.03
24.i-20.ii	268	268	3,588	2,814	2,347	2,216	2	0.09	6,203	5,298	2	0.04
21.ii-20.iii	17	17	2,080	2,080	1,075	1,075	3,172	3,172
21.iii-17.iv	10	10	1,344	1,255	487	470	1,841	1,735
18.iv-15.v	7	7	144	138	197	197	348	342
16.v-12.vi	8	8	936	936	866	866	1,810	1,810
13.vi-10.vii	20	20	2,056	2,056	1,515	1,515	3,591	3,591
11.vii-8.viii	142	140	1	0.7	1,400	1,064	1	0.09	966	878	2,508	2,082	2	0.07
9.viii-4.ix	496	496	12	2.4	1,235	1,235	1	0.08	682	682	2,413	2,413	13	0.59
5.ix-2.x	1,259	1,259	15	1.2	3,135	3,135	2	0.06	1,390	1,390	5,787	5,784	17	0.29
3.x-30.x	5,152	5,149	54	1.1	3,746	2,728	2	0.07	3,739	2,645	1	0.04	12,637	10,522	57	0.57
31.x-27.xi	3,303	3,291	40	1.2	5,780	3,226	1	0.03	5,026	3,495	14,109	10,012	41	0.41
28.xi-24.xii	688	680	6	0.9	3,781	2,566	1	0.04	1,736	1,601	6,205	4,847	7	0.14
1934																
-22.i	26	13	4,511	1,295	1,482	559	6,019	1,867
23.i-21.ii	46	46	5,300	2,533	785	588	6,131	3,167
22.ii-22.iii	43	43	4,325	2,155	343	251	4,711	2,449
23.iii-31.iii	0	0	649	485	15	15	664	500
Total ..	12,635	12,594	131	1.04	49,698	34,041	8	0.02	35,429	27,238	9	0.03	97,762	73,873	148	0.20

* Cat. = Catch, Diss. = Dissections, Inf. = Infections, Rate = Sporozoite rate per cent of dissections.

TABLE V.

Total catches and dissections of salivary glands of all other species.

Species.	Catches.	Dissections.
<i>A. subpictus</i>	23,475	8,394
<i>A. hyrcanus</i> (var. <i>nigerrimus</i>)	15,970	8,274
<i>A. vagus</i>	9,966	3,722
<i>A. culicifacies</i>	1,987	1,946
<i>A. barbirostris</i>	2,248	380
<i>A. jamesi</i>	1,322	1,011
<i>A. aconitus</i>	1,094	928
<i>A. minimus</i>	391	340
<i>A. ramsayi</i>	36	36
<i>A. tessellatus</i>	4	4
<i>A. theobaldi</i>	3	3
<i>A. karwari</i>	2	2
	56,498	25,040
Add totals for		
<i>A. annularis</i>	49,698	34,041
<i>A. pallidus</i>	35,429	27,238
<i>A. philippinensis</i>	12,635	12,594
GRAND TOTAL	154,260	98,913

TABLE VI.

Prevalence of less common species of Anophelines.

Species.	Present.	Period of greatest prevalence.	Largest catch in 4-week period.	Average catch in 4-week period.
<i>A. culicifacies</i>	All year	April-May	561	90
<i>A. barbirostris</i>	Aug.-Mar.	Sept.	770	110
<i>A. jamesi</i>	All year	Nov.-Dec.	649	60
<i>A. aconitus</i>	"	Jan.	186	49
<i>A. minimus</i>	"	Jan.-Feb.	98	17
<i>A. ramsayi</i>	July-Feb.	Dec.-Jan.	12	2
<i>A. tessellatus</i>	Dec.-Jan.	"	2 each	..
<i>A. theobaldi</i>				
<i>A. karwari</i>				

treatment in the form of plasmoquine was given to the people in these villages during the period under consideration. The people in the Peripheral Villages were taking a certain amount of quinine during this period but not to an extent which could in any way be considered as materially affecting the parasite or gametocyte rate. In Table VII, a case is counted as a 'new' fever case only when it was reported for the first time. Second or subsequent reports of fever on the same person are not included in this table, except in those cases

TABLE VII.

Fever rates, malaria rates, and gametocyte rates, per cent of population for the Peripheral Villages (where no anti-gametocyte treatment took place), and sporozoite rates, per cent of A. philippinensis dissected from these villages, for 4-weekly periods from 11th July, 1933 to 21st February, 1934.*

4-week period.	New fever cases.	Number with parasites.	Number with gametocytes.	Fever rate per cent of population.	Parasite rate per cent of population.	Gametocyte rate per cent of population.	Sporozoite rate per cent in <i>A. philippinensis</i> .†
1933.							
11.vii-8.viii	82	18	0	7.0	1.5	0.0	0.89
9.viii-4.ix	197	76	18	16.9	6.6	1.55	2.88
5.ix-2.x	135	78	4	11.6	6.7	0.34	1.21
3.x-30.x	166	83	9	14.3	7.1	0.77	1.00
31.x-27.xi	65	32	9	5.6	2.7	0.77	1.25
28.xi-24.xii	13	4	1	1.1	0.3	0.09	1.00
1934.							
-22.i	2	1	1	0.17	0.08	0.09	0.00
23.i-21.ii	22	13	13	1.9	1.1	1.1	0.00
TOTAL ..	682	305	55	58.6	26.2	4.75	

* All the population of Ruppur and Manoharpur, and 79 families, or 417 persons, in Surul, making a total population of 1,162.

† These rates differ from those in Table IV, because the latter are based on dissections from all the surveyed villages, while the former on those from the Peripheral Villages only.

where no parasites were found at the primary attack, but were found in a subsequent one, in which case the latter attack is included.

In this way we have tried to reduce the counting of relapsing cases to a minimum and have thereby probably omitted a good many cases of primary malaria infection. Table VII, therefore, must represent the minimum fever and malaria incidence in the Peripheral Villages. The exclusion from the record, of fever cases from which no blood slide was obtained, has already been noted above.

From this table it can be seen that the greatest 'new' fever rate (16.9 per cent of the population) occurred about mid-August, but that the greatest malaria parasite rate (7.1 per cent of the population), which might better be called the 'primary malaria case rate', occurred about mid-October. The higher fever rate in August can be accounted for by the great number of respiratory infections occurring during the height of the monsoon.

From Table VII it can also be seen that the greatest sporozoite rate in *A. philippinensis* (2.88 per cent) occurred about mid-August and that this was also the period when the gametocyte rate was highest (1.55 per cent) based on the blood slides from that part of the population reporting fever during the autumn epidemic.

One would be tempted to recognize in these figures a close correlation between the highest sporozoite rate of *A. philippinensis* and the greatest prevalence of gametocytes in the population, were it not for the fact that the gametocyte rates in Table VII are based on slides taken only from persons reporting fever. That the gametocyte prevalence of this part of the population may not represent the gametocyte prevalence of the population as a whole is indicated in Table I, where it can be seen that in April and May 1933, 21·7 per cent of the children (based on blood slides from at least 54 per cent of all the children in the surveyed population of the Peripheral Villages) had gametocytes in their blood. In October 1933, the figure was 18·9 per cent, in December 1933, 31·7 per cent, and in March 1934, 19·1 per cent. There being 601 children in the surveyed population of these villages according to our census of March 1933, these figures would represent a gametocyte rate in the entire population of 1,162 persons, of 11·2 per cent in April and May 1933, 9·7 per cent in October, 16·3 per cent in December, and 9·8 per cent in March 1934. Since gametocytes, although found more frequently in the blood of children especially in those below 2 years of age, may also be found in the blood of adults, these figures must represent the minimum gametocyte prevalence in the population for the period under consideration. In any event it would appear that the greatest gametocyte rate in the entire population was in December 1933, and that at no time in the year, when a parasite survey was made, were there gametocytes in less than 9·7 per cent of the population.

The conclusion to be made would therefore seem to be that the high sporozoite rate in *A. philippinensis* in mid-August 1933 represented its *period of greatest infectivity* rather than its access to the greatest number of gametocytes in the population. This conclusion is borne out by the fact that Table IV shows that *A. philippinensis* became much more numerous in mid-October than it was in mid-August, and yet its sporozoite rate was only half as high (1·1 per cent) during the former as during the latter period.

In any event Table VII shows *A. philippinensis* to be an effective carrier of malaria during the autumn epidemic of 1933, since its highest sporozoite rate preceded the peak of the 'primary malaria case rate' by a period of not more than 8 weeks. Four weeks following the disappearance of sporozoites from *A. philippinensis* in December 1933, the parasite rate or primary malaria case rate in the population dropped to about 1 per cent of its peak value of 7·1 per cent in mid-October.

Table IV shows that *A. annularis* was also a carrier of considerable importance in the surveyed area in the summer and autumn of 1933. Its highest 4-weekly sporozoite rates (0·09 per cent and 0·08 per cent) fell between 11th July and 4th September. During this period it was about 3 times as numerous as *A. philippinensis* but about 22 times less frequently infected, making it about 7 times less effective as a carrier of malaria.

A. pallidus (Table IV) was found infected during the autumn of 1932 to about the same degree as *A. annularis* in the autumn of 1933. Hence these two species were apparently responsible for a certain amount of the transmission of malaria during these years of the survey, but to a much less degree than was *A. philippinensis*.

Table IV might be regarded as indicating that *A. philippinensis* was not as effective a carrier in the autumn of 1932 as it was in the autumn of 1933, since

it was found infected over a much shorter period of time and its prevalence in absolute numbers was far less. But, as will shortly be pointed out, probably between 7 and 23 times fewer *A. philippinensis* were being captured during 1932 than it would have been possible to capture had the catches been done at night instead of by day.

Neither *A. philippinensis* nor *A. pallidus* was found infected during the early months of 1934 (Table IV). *A. philippinensis* was not numerous at that time, but *A. pallidus* was still quite prevalent although decreasing. In the corresponding months of the preceding year, *A. pallidus* seems to have been more prevalent, and three infections were found in it at that time. *A. annularis*, although very numerous, was not found infected during that season in either year. From this it would seem that *A. pallidus* was chiefly concerned in the spring epidemic of 1933. The survey gave no clue as to which anopheline was concerned in producing the primary infections in the spring epidemic of 1934, but it was probably not *A. philippinensis*.

Table I shows gametocytes to have been present in about 20 per cent of the children of the Peripheral Villages during the spring of 1933 and 1934. *A. annularis* was much more prevalent during this season (Table IV) than it was during its period of highest sporozoite rate, July to September 1933. The highest gametocyte rate in the population is recorded as having occurred in December 1933 (Table I, 31.7 per cent), a month when *A. annularis* had attained its greatest prevalence, but only one infected specimen of this species was found during that month. Here, as in the case of *A. philippinensis*, we would seem to have an expression of the fact that the period of greatest infectivity of *A. annularis* did not correspond to its period of greatest prevalence nor to the period of greatest gametocyte prevalence in the population. Judging from the sporozoite rates of *A. pallidus* in the autumn of 1932 (Table IV), the same observation may be made regarding this anopheline also. This anopheline, however, was found infected during January and February 1933. *A. philippinensis* and *A. annularis* were not found to be infected in these months of either year. The question therefore arises as to whether *A. annularis* and *A. philippinensis* are capable of being infected with gametocytes during the cold months of the year in the area under consideration? Our survey would seem to indicate that they are not.

Collections of larvae.

The collections of larvæ for 4-weekly periods are shown in Table VIII. Since the mortality of the larvæ when being bred out into adults was between 60 per cent and 80 per cent of those collected, the figures for the first 12 weeks shown in the table indicate only a proportion of the actual numbers of larvæ collected. They may, however, give a more accurate idea of the number of larvæ that survived and eventually reached the adult stage in nature than do the figures in the second part of the table, which are those of identifications of 4th stage larvæ. The collections were not sufficiently numerous, nor were they extended over a long enough period, to permit any conclusions to be drawn regarding the relation between larva and adult prevalence of the different species. The following points of interest in this connection may be noted :—

(1) *A. annularis*.—Its larvæ were being recovered with decreasing frequency during the last three months of the year, while the number of adults (Table IV)

was increasing. This may be an indication of the prolongation of the pupal stage of this mosquito during this season.

TABLE VIII.

Numbers of anopheline larvae found in Central Villages, by 4-weekly periods, from 14th June to 27th December, 1932.

Date.	Technique employed.	<i>hyrcanus</i> var. <i>nigerrimus</i> .	<i>barbirostris</i> .	<i>subpictus</i> .	<i>vagus</i> .	<i>pallidus</i> .	<i>annularis</i> .	<i>philippinensis</i> .	<i>culicifacies</i> .	<i>aconitus</i> .	<i>minimus</i> .	Total.
1932.												
14.vi-12.vii	Bred	10	0	127	2	26	28	4	8	1	0	206
13.vii-8.viii	into	273	43	237	31	104	113	19	27	15	1	863
9.viii-6.ix	adults.	652	66	70	47	23	37	16	6	15	0	932
7.ix-3.x	Identi-	392	119	67	51	17	21	20	0	4	4	695
4.x-1.xi	fied	433	253	41	113	83	19	15	4	3	1	965
2.xi-29.xi	as 4th	348	490	275	220	141	16	9	0	0	0	1,499
30.xi-27.xii	stage	145	859	497	335	134	10	13	1	0	0	1,994
	larvæ.											
TOTAL ..		2,253	1,830	1,314	799	528	244	96	46	38	6	7,154

A. jamesi, 5 larvæ, one in every month except September, October, and December.

A. ramsayi, 10 larvæ, one or two in every month except June and October.

A. tessellatus
A. theobaldi
A. karwari } no larvæ found.

(2) *A. barbirostris*.—This mosquito was never very prevalent (Table V) as indicated by adult catches in houses and cowsheds. Its greatest prevalence as an adult was in September. Its larvæ showed an increasing frequency from September onward, until they constituted nearly 50 per cent of the total catch of larvæ in December. Apparently adult catches in houses and cowsheds did not give a representative idea of the prevalence of this anopheline. It was, therefore, probably wild.

Breeding places.

A. philippinensis, *A. annularis* and *A. pallidus* were all found to breed in the same type of water, viz., clean, partially shaded pools containing a good deal of aquatic vegetation. Their larvæ were never found in shallow, temporary collections of water, nor in pools in rice fields. Larvæ of *A. culicifacies* were occasionally found in the same type of breeding places as the three species mentioned above, but more frequently in green algæ in the slowly moving water along the edges of the Kopai River. *A. minimus*, *A. aconitus*, *A. jamesi* and *A. ramsayi* were found in the same type of breeding place as the first three named above. *A. subpictus*, *A. vagus*, *A. barbirostris* and *A. hyrcanus* (var. *nigerrimus*) were found in collections of water of almost every kind.

II. COMPARATIVE ANTHROPOPHILISM AND ZOOPHILISM, COMPARISON OF FEEDING PREFERENCES, AND DIURNAL AND NOCTURNAL ACTIVITIES, OF *A. PHILIPPINENSIS* AND OTHER ANOPHELES.

ANTHROPOPHILISM IN *A. PHILIPPINENSIS* AND ITS COMPARISON IN THIS RESPECT TO OTHER ANOPHELES.

A. philippinensis was observed to have a decided preference for human habitations rather than those of cattle, since this species could be captured at least 8.6 times as frequently in houses as in cowsheds. Under the same conditions of catching, on the other hand, *A. hyrcanus* (var. *nigerrimus*) could be captured 3.1 times as frequently in cowsheds as in houses. The relative prevalence in houses and cowsheds of the most common anophelines in the region is shown in Table IX.

TABLE IX.

Comparative anthropophilism and zoophilism in certain anophelines, as shown by their relative prevalence in houses and cowsheds.

Species.	TOTAL.		HOUSE.		COWSHED.		Ratio : Per cent of house catch/ Per cent of cowshed catch.
	No.	Per cent of total catch.	No.	Per cent of total house catch.	No.	Per cent of total cowshed catch.	
<i>A. philippinensis</i>	12,635	8.19	12,253	10.07	382	1.17	8.6/1
<i>A. hyrcanus</i> (var. <i>nigerrimus</i>).	15,970	10.06	8,755	7.19	7,215	22.13	1/3.1
<i>A. annularis</i> ..	49,698	32.20	40,581	33.35	9,117	27.96	1.1/1
<i>A. pallidus</i> ..	35,429	22.90	29,150	23.96	6,279	19.26	1.4/1
<i>A. subpictus</i> ..	23,475	15.20	17,607	14.47	5,868	18.00	1/1.2
<i>A. vagus</i> ..	9,966	6.46	7,565	6.22	2,401	7.36	1/1.2
<i>A. culicifacies</i> ..	1,987	1.29	1,739	1.43	248	0.76	1.8/1
<i>A. aconitus</i> ..	1,094	0.71	956	0.78	138	0.42	1.8/1
<i>A. minimus</i> ..	391	0.25	326	0.27	65	0.20	1.2/1
All others ..	3,615	2.34	2,749	2.26	866	2.65	
TOTAL ..	154,260	100	121,681	100	32,579	100	

There can be no question of accounting for the differences in house and cowshed prevalence of the various species, noted in Table IX, on the basis of a difference in accessibility of houses and cowsheds to the ingress of flying insects. The catches were made at random in the villages, and selective catching was, of course, impossible. Besides, each household in these villages is contained within a mud-walled compound in one part of which is the dwelling and in another part the cowshed. Hence, houses and cowsheds are nearly equally distributed throughout the village.

With the exception of *A. culicifacies*, *A. aconitus* and *A. minimus*, the collections were sufficiently large to make the differences in prevalence in houses

and cowsheds, noted in Table IX, statistically significant. The probability of even the smallest of these differences having occurred by chance is of a very low order. It might be questioned, however, whether the smaller differences have biological significance as representing species preference for human or bovine habitations. It might be questioned, for instance, whether, in the case of *A. annularis*, a ratio of house to cowshed prevalence of 1.1/1, even when based on nearly 50,000 caught specimens and having statistical significance, really indicates a definite shade of preference on the part of this anopheline for human habitations. But a ratio of house/cowshed prevalence as great as 8.6/1, such as obtained with *A. philippinensis* and based on over 12,000 collections, must surely indicate a definite preference on the part of this anopheline for human dwellings. A ratio in the other direction of 1/3.1 based on over 15,000 collections must indicate a preference on the part of *A. hyrcanus* (var. *nigerrimus*) for cowsheds.

If all of the statistically significant differences in house and cowshed prevalence shown in Table IX may be regarded as having biological significance also, then it is of interest to note that the three closely related species, viz., *A. philippinensis*, *A. annularis* and *A. pallidus*, all have a preference for human habitations. *A. philippinensis* exhibits this preference to a much greater degree than do the other two. Does this represent an evolutionary adaptation to the human host on the part of *A. philippinensis*?

It is of interest also to note that *A. culicifacies*, *A. aconitus* and *A. minimus*, species which have been incriminated as carriers of malaria in other parts of India, all seem to have been more prevalent in houses. It is true that the collections of these species in the surveyed area were not large, but is it not rather significant that they all seemed to have exhibited their preferences in the same direction? *A. hyrcanus*, *A. subpictus* and *A. vagus*, on the other hand, none of which have ever been incriminated as carriers of malaria in India, all show a definite preference for cowsheds. Might we suggest that herein may lie a clue to the mystery of what makes some species of *Anopheles* carriers of malaria and others not?

COMPARATIVE DIURNALISM AND NOCTURNALISM IN HOUSES AND COWSHEDS.

In respect to their comparative prevalence in houses and cowsheds by day and by night, *A. philippinensis* and *A. hyrcanus* (var. *nigerrimus*) were again found to be diametrically opposite. The former could be captured 6.9 times as frequently by night as by day, and the latter 3.5 times as frequently by day as by night. Again, the figures are large enough to give these differences great statistical significance. The prevalence by day and by night in houses and cowsheds for all of the most common anophelines in the region is shown in Table X. It should be noted that in these villages the cattle leave the cowsheds by day.

A. subpictus, which exhibited only a minor shade of preference for bovine rather than human habitations, was like *A. hyrcanus* (var. *nigerrimus*) in showing definite diurnalism in houses and cowsheds, since it could be captured 3.4 times as frequently, by day as by night. *A. annularis* and *A. pallidus*, on the other hand, are seen to be more like *A. philippinensis* in their greater night prevalence, but to a much less marked degree.

TABLE X.

Comparative diurnal and nocturnal habits of certain anophelines as shown by their day and night prevalence in houses and cowsheds, and in both places considered together.

Species...	DAY.						NIGHT.						Ratio : Day percentage Night percentage
	HOUSES.		COWSHEDS.		TOTAL.		HOUSES.		COWSHEDS.		TOTAL.		
	Number.	Per cent of total house day catch.	Number.	Per cent of total cowshed day catch.	Number.	Per cent of total day catch.	Number.	Per cent of total house night catch.	Number.	Per cent of total cowshed night catch.	Number.	Per cent of total night catch.	
<i>philippinensis</i>	2,232	3.20	273	0.98	2,505	2.58	10,021	19.23	109	2.29	10,130	17.81	1/69
<i>hyrcanus</i>	7,491	10.76	6,185	22.21	13,676	14.03	1,264	2.43	1,030	21.65	2,294	4.03	35/1
<i>annularis</i>	19,663	28.26	7,311	26.26	26,974	27.69	20,918	40.14	1,806	37.96	22,724	39.96	1/1.4
<i>pallidus</i>	17,151	24.65	4,892	17.57	22,043	22.62	11,999	23.03	1,387	29.16	13,386	23.54	1/1
<i>subpictus</i>	14,306	20.56	5,737	20.60	20,043	20.57	3,301	6.33	131	2.75	3,432	6.03	34/1
<i>vagus</i>	4,967	7.13	2,266	8.14	7,233	7.42	2,598	4.98	135	2.84	2,733	4.80	15/1
<i>culicifacies</i>	1,354	1.94	212	0.76	1,566	1.61	385	0.73	36	0.75	421	0.74	22/1
<i>aconitus</i>	614	0.84	99	0.35	713	0.73	342	0.66	39	0.82	381	0.67	1/1
<i>minimus</i>	185	0.26	54	0.19	239	0.25	141	0.27	11	0.23	152	0.27	1/1
All others	1,608	2.31	804	2.88	2,412	2.47	1,141	2.19	62	1.30	1,203	2.11	
Total ..	69,571	100	27,833	100	97,404	100	52,110	100	4,746	100	56,856	100	

In Table XI the difference in prevalence as between 'early night' (9 to 11 p.m.) and 'late night' (3 to 5 a.m.) are shown. It would be expected that a predominantly night-prevailing mosquito like *A. philippinensis* would be found more frequently late at night than in the early night. Table XI shows

TABLE XI.

Comparative 'early night' and 'late night' habits of certain anophelines in houses and cowsheds considered together.

Species.	TOTAL NIGHT CATCH.		EARLY NIGHT.		LATE NIGHT.		Ratio : Early night percentage/ Late night percentage.
	No.	Per cent of total night catch.	No.	Per cent of total early night catch.	No.	Per cent of total late night catch.	
<i>A. philippinensis</i>	10,130	17.82	3,580	12.17	6,550	23.87	1/2
<i>A. hyrcanus</i> ..	2,294	4.03	1,333	4.53	961	3.50	13/1
<i>A. annularis</i> ..	22,724	39.97	11,337	38.54	11,387	41.50	1/1.1
<i>A. pallidus</i> ..	13,386	23.52	7,893	26.80	5,493	20.02	13/1
<i>A. subpictus</i> ..	3,432	6.03	2,508	8.52	924	3.36	25/1
<i>A. vagus</i> ..	2,733	4.82	1,641	5.58	1,092	3.98	14/1
<i>A. culicifacies</i> ..	421	0.74	309	1.05	112	0.41	26/1
<i>A. aconitus</i> ..	381	0.67	145	0.49	236	0.88	1/1.8
<i>A. minimus</i> ..	152	0.27	84	0.28	68	0.25	1/1
All others ..	1,203	2.11	597	2.03	606	2.21	
TOTAL ..	56,856	100	29,427	100	27,429	100	

the ratio to be 2/1. On the other hand, a mosquito found more frequently during the day than during the night ought also to be more frequent in the early night than in the late night. This expectancy is confirmed in Table XI for *A. subpictus* and *A. hyrcanus* (var. *nigerrimus*). Table XI, however, gives only a very inadequate idea of how great the difference in density of *A. philippinensis* between the early and late hours of night may be at certain times. For example, during the first week of November 1933 the same number of collectors working in the villages of Surul and Ruppur, for the same number of hours, early night and late night, caught 46 *A. philippinensis* during the former and 1,025 during the latter period. While these figures are more extreme than those usually encountered in our records, they indicate how this mosquito may at times crowd into the houses during the later hours of the night.

Since the catches grouped in Tables IX, X and XI are comparable only when related to the total catch for each group and not on the basis of the absolute numbers, which are given only to show the size of catch on which the comparisons are based, it might be argued that the differences noted might be due to differences in the technique of catching, or to seasonal variations in prevalence of the anophelines concerned, or to factors other than actual differences in species preference for special times and places. It would therefore be of interest to see if the relative differences noted in these tables would be confirmed if absolute numbers of catches, representing the same amount of

labour, could be compared. Our data offers such a comparison. For a period of 8 weeks, from 31st October to 24th December, 1933, we kept a separate record of the following catches in four villages, viz., the three Peripheral Villages and Lohargar: (1) day (7 to 9 a.m.); (2) early night (9 to 11 p.m.); (3) late night (3 to 5 a.m.); in (4) houses; and (5) cowsheds. Each group constitutes the catch of one man working two hours daily at the times specified in houses and cowsheds in the 4 villages. Since the catching was done by our most experienced men, between whom there were not any great differences in skill and speed in catching, we feel that the numbers of catches in the same length of time are comparable.

The results of the catches, made under these conditions, of *A. philippinensis*, *A. pallidus* and *A. annularis*, are shown in Table XII. The house and cowshed

TABLE XII.

Comparison of house and cowshed prevalence, diurnal and nocturnal habit, and early night and late night prevalence, of A. philippinensis, A. annularis and A. pallidus, on basis of absolute numbers of catches when an equal period of time was spent in catching in houses and cowsheds during the day (7 to 9 a.m.), during the early night (9 to 11 p.m.) and during the late night (3 to 5 a.m.), for 8 weeks from 31st October to 24th December, 1933.

Species.	Place.	Time.	Catch.	Ratio : House Cowshed	Day \times 2 Night	Early night Late night
<i>A. philippinensis</i>	H.	Day	26			
		E. N.	253			
		L. N.	1,406	1,685 or 167	38×2 or 1	264 or 1
	C.S.	Day	12	101 or 1	1,748 or 23	1,484 or 54
		E. N.	11			
		L. N.	78			
<i>A. annularis</i> ..	H.	Day	413			
		E. N.	1,103			
		L. N.	1,323	2,839 or 16	$1,381 \times 2$ or 1	1,435 or 1
	C.S.	Day	968	1,728 or 1	3,186 or 11	1,751 or 12
		E. N.	332			
		L. N.	428			
<i>A. pallidus</i> ..	H.	Day	247			
		E. N.	632			
		L. N.	529	1,408 or 15	757×2 or 1	830 or 11
	C.S.	Day	510	947 or 1	1,598 or 1	768 or 1
		E. N.	198			
		L. N.	239			

Total number of catching hours—192.

H = House; C. S. = Cowshed; E. N. = Early night; L. N. = Late night.

catches in this table, representing the same length of catching time, are comparable on an equal basis. So are the early night and late night catches. Since the day catch, however, represents only 2 hours and the night catch 4 hours, the former must be multiplied by 2 before being compared with the latter.

Comparison of Table XII with Tables IX, X and XI shows that the first confirms in every respect the differences observed in the others for the three carrier anophelines. In fact, Table XII shows that these differences are even greater when based on comparable numbers of catches than when based on numbers relative to the total catches. For instance, from Table IX, *A. philippinensis* is seen to have been 8.6 times as prevalent in houses as in cowsheds; from Table X, 6.9 times as prevalent by night as by day; and from Table XI, twice as prevalent late night as early night. From Table XII, these multiples are 16.7, 23, and 5.4, respectively. In regard to *A. annularis* and *A. pallidus* also, Table XII is consistent with and confirms Tables IX, X and XI. The same is true also for *A. vagus* and *A. hyrcanus*, the numbers for which it is not necessary to include in the table. *A. subpictus* and other species were not numerous enough during this period to make comparisons reliable.

The ratios between percentages of total catch, for house catches/cowshed catches, day catches/night catches, and early night catches/late night catches, noted in Tables IX, X and XI, are shown in parallel columns in Table XIII

TABLE XIII.

Summary of ratios of house and cowshed prevalence, diurnalism and nocturnalism in houses and cowsheds, and early and late night prevalence in houses and cowsheds, in those anophelines in which the numbers of catches exceeded 9,000 during the entire survey.

Species.	House Cowshed	Day Night	Early night Late night	Suggested conclusions.
<i>A. philippinensis</i>	9 : 1	1 : 7	1 : 2	Definitely anthropophilic. Leaves houses by day. Most numerous in late hours of night.
<i>A. hyrcanus</i> (var. <i>nigerrimus</i>).	1 : 3	3 : 1	1.3 : 1	Definitely zoophilic. Flies into cowsheds by day, and tends to leave them by night. Has it another source of nourishment than either man or cattle?
<i>A. annularis</i> ..	1.1 : 1	1 : 1.4	1 : 1.1	Tends to show the same characters as <i>A. philippinensis</i> but to a much less marked degree.
<i>A. pallidus</i> ..	1.4 : 1	1 : 1	1.3 : 1	Like <i>A. annularis</i> and <i>A. philippinensis</i> except that it is more domestic, in that its movements between day and night are not so marked.
<i>A. subpictus</i> ..	1 : 1.2	3.4 : 1	2.5 : 1	Less zoophilic than <i>A. hyrcanus</i> (var. <i>nigerrimus</i>) and shows a slightly greater degree of diurnalism.
<i>A. vagus</i> ..	1 : 1.2	1.5 : 1	1.4 : 1	Same characters as <i>A. subpictus</i> but to a less marked degree.

for the anophelines the total catch of which during the period of survey was more than 9,000. In this table are suggested certain conclusions which may be drawn from these ratios regarding the habits of the anophelines.

The effect which these habits have on the absolute numbers of adult anophelines, captured when the technique of capture is varied from time to time, is seen in Graphs 1 and 5, the graphs of absolute numbers of catches of some of the most common anophelines by 4-weekly periods during the survey. At the beginning of this paper it was pointed out that until 31st May, 1933, most of the catches were made during the day in houses and cowsheds, whereas after that date emphasis was placed upon night catches in houses. Dividing Graphs 4 and 5 into two parts at the end of May 1933, we can compare the curves for each species of anophelines for the same season in the two parts. *A. annularis* and *A. pallidus* exhibited no great differences between their prevalences in houses and cowsheds or by day and night (Tables IX and X). Hence we should expect the graphs of the catches of these species to exhibit approximately the same modal and minimal points on either side of the dividing line in May 1933. And they do. *A. annularis*, which is seen in Graph 4 to have increased in prevalence from September 1932 to a peak in February and March 1933, showed approximately the same rise in the following year. *A. pallidus* repeats its curve in approximately the same way in the two parts of the survey. In other words, the difference in the technique of capture in the two parts made very little difference in the numbers of these species captured. The same may be said of *A. subpictus*, which, although more prevalent by day than by night, did not greatly prefer cowsheds to houses. But with *A. philippinensis* and *A. hyrcanus* (var. *nigerrimus*) the case is seen to be definitely otherwise. In the first part of the survey, day catches were done much more frequently than night catches, and cowshed catches were made quite as frequently as house catches. This technique, therefore, made it appear that *A. philippinensis* (having a great density in houses by night and fully as great a scarcity in houses and particularly in cowsheds by day) was a comparatively rare mosquito in the district. *A. hyrcanus* (var. *nigerrimus*), on the other hand, a frequenter of cowsheds by day, seemed to be rather numerous. When the technique was changed, so that more captures were made in houses and during the night, the appearances were reversed, and *A. philippinensis* then appeared to have been very numerous, and *A. hyrcanus* (var. *nigerrimus*) comparatively rare, as seen in the second part of Graph 5.

III. A NOTE ON THE EFFECT OF PARTIAL PLASMOQUINIZATION OF THE CENTRAL VILLAGES.

From July 1933 until February 1934 a plasmoquine experiment was carried on in the Central Villages. The object was to see if partial 'plasmoquinization' of those persons most likely to harbour gametocytes would have any noticeable effect in reducing the incidence of malaria.

The procedure which was followed is shown in Table XIV.

Drug distributions were made by members of our own staff and, in the majority of instances, the drugs were swallowed at the time of distribution. In Islampur practically 100 per cent of the persons indicated in Table XIV co-operated. In the other three villages about 80 per cent of the children, and about 60 per cent of the adults indicated in Table XIV, co-operated.

TABLE XIV.

Drug.	CLASSES OF PERSONS TO WHOM THE DRUGS WERE GIVEN, AND TIMES OF DISTRIBUTION.			
	Children 0-4.	Children 5-14.	Persons, 14 and over, with enlarged spleen or with history of chronic fever during the past year.	Persons reporting fever to daily fever reporters.
Plasmoquine Simplex	(1) June 1933, $\frac{1}{4}$ grain in one week. (2) 15th Oct.-21st Feb., $\frac{1}{12}$ grain weekly.	(1) June 1933, $\frac{1}{2}$ grain in one week. (2) 15th Oct.-21st Feb., $\frac{1}{6}$ grain weekly.	(1) June 1933, $\frac{1}{2}$ grain in one week.	11th July-21st Feb. Over 5 yrs.— $\frac{1}{6}$ grain daily for 5 days. Under 5 yrs.— $\frac{1}{12}$ grain daily for 5 days.
Quinine Sulphate	(1) June 1933, 5 grains daily for 3 weeks. (2) 15th Oct.-21st Feb., $2\frac{1}{2}$ grains weekly.	(1) June 1933, 10 grains daily for 3 weeks. (2) 15th Oct.-21st Feb., 5 grains weekly.	(1) June 1933, 15 grains daily for 3 weeks.	11th July-21st Feb. Over 14 yrs.— 15 grains daily for 5 days. 5-14 yrs.—10 grains daily for 5 days. Under 5 yrs.— 5 grains daily for 5 days.

Children being treated as fever cases were omitted from weekly distributions of drugs during their fever treatments.

Among children, the least co-operation was obtained in the case of babies under 2 years of age whose parents did not wish to have them given drugs of any kind.

In order to obtain a basis for comparison, daily fever reports and blood slides from fever cases were obtained from the Peripheral Villages in the same manner as in the Central Villages, but no plasmoquine and only a very small amount of quinine was distributed in the former.

Spleen and parasite surveys were made among the children of the Central and Peripheral Villages in April-May, and October 1933, and in March 1934, and a parasite survey was made in December 1933.

The results were as follows:—

(1) As can be seen in Table I, between April-May 1933 and March 1934, the spleen rate of the Central Villages rose from 66.8 per cent to 87.5 per cent, an increase of 31 per cent. During the same period in the Peripheral Villages, the spleen rate rose from 48.0 per cent to 59.2 per cent, an increase of 23 per cent.

(2) Between these dates, the average enlarged spleen in the Central Villages decreased by about 1 cm., whereas in the Peripheral Villages it increased by about 0.5 cm. (Table I).

(3) In the Central Villages the parasite rate among the children underwent a reduction from 31.2 per cent to 17.3 per cent, a decrease of 45 per cent, whereas in the Peripheral Villages there was a decrease in the parasite rate of 21 per cent (42.4 per cent to 33.3 per cent). In the Central Villages, the gametocyte rate among the children fell from 13.5 per cent to 5.0 per cent, a reduction of 63 per cent whereas in the Peripheral Villages there was a reduction in the gametocyte rate of 9 per cent (21.7 per cent to 19.1 per cent).

(4) The new fever rates and the new malaria case rates or parasite rates per cent of population, derived from the daily fever reports of the Central Villages, are shown in Table XV. These are comparable with the equivalent

TABLE XV.

Fever rates and malaria rates, per cent of population, for the Central Villages, by 4-weekly periods, from 11th July, 1933 to 21st February, 1934. Compare with equivalent figures for Peripheral Villages in Table VII.*

4-week period.		New fever cases.	Number with parasites.	Fever rate per cent of population.	Parasite rate per cent of population.
1933	11.vii-8.viii ..	61	15	4.3	1.0
	9.viii-4.ix ..	106	45	7.5	3.2
	5.ix-2.x ..	77	47	5.4	3.3
	3.x-30.x ..	84	30	5.9	2.1
	31.x-27.xi ..	77	32	5.4	2.2
	28.xi-24.xii ..	23	13	1.6	0.9
1934	-22.i ..	18	9	1.2	0.6
	23.i-21.ii ..	18	5	1.2	0.4
TOTAL ..		464	196	32.5	13.7

* Population, 1,420.

figures shown for the Peripheral Villages in Table VII. Comparing these two tables it can be seen that for the entire period the fever rate for the Central Villages was 32.5 per cent as compared with 58.6 per cent in the Peripheral Villages, and the parasite rate for the Central Villages was 13.7 per cent as compared with 26.2 per cent in the Peripheral Villages.

(5) Between 11th July and 24th December, 1933, there were 1,450 *A. philippinensis* dissected from the Central Villages. Of these anophelines, 13 were found infected, making a sporozoite rate of 0.89 per cent. In the same period, from the Peripheral Villages 9,565 *A. philippinensis* were dissected, of which 115 were infected, making a sporozoite rate of 1.20 per cent. The differences in these sporozoite rates are statistically significant, corresponding to deviation/ σ of 2.9, an event which, according to Pearson (1914), would occur by chance only once in 267 trials.

Of *A. annularis*, 5,821 were dissected from the Central Villages and 2 were found infected, making a sporozoite rate of 0.038 per cent, and from the Peripheral Villages, 8,133 were dissected, of which 6 were found infected, making a sporozoite rate of 0.074 per cent. The one specimen of *A. pallidus* found infected during this period came from the Central Villages.

Hence it would appear that the sporozoite rate among two of the carrier anophelines was less in the Central than in the Peripheral Villages during the period when plasmoquine and quinine were being distributed. At the same time it should be observed that, according to Table I, in April and May 1933, before any antigametocyte drugs were distributed, the gametocyte rate was lower in the Central Villages than in the Peripheral Villages.

(6) When the time spent in catching mosquitoes in the Central Villages was equal to that spent at corresponding hours of the day and night in the Peripheral Villages, there were considerably fewer *A. philippinensis* captured in the former than in the latter. Between 11th July and 24th December, 1933, for equal and corresponding catching times, there were 1,238 *A. philippinensis* captured in the Central Villages, and 4,136 in the Peripheral Villages. From this it would appear that the chief carrier was about 3 times as prevalent in the Peripheral Villages as in the Central Villages. This fact might afford a partial explanation of the higher malaria rates in the Peripheral Villages.

It must also be remembered that there was a dispensary in the Central Villages, treating for the most part persons from these villages. In this dispensary, quinine but not plasmoquine was given out.

It is difficult to place an interpretation on these results.

In favour of the argument that the antimalaria measures, including the distribution of plasmoquine, had effects are :

- (1) The lesser fever and malaria incidence in the Central Villages.
- (2) The greater degree of reduction in the parasite and gametocyte rates among the children of the Central Villages.
- (3) The lesser sporozoite rate among *A. philippinensis* and *A. annularis* in the Central Villages.

Against the argument that the antimalaria measures had effect is the greater increase in the spleen rate in the Central than in the Peripheral Villages. In fact, it would have been expected that the spleen rate would have shown some reduction between the spring of 1933 and the spring of 1934 in the Central Villages even though it increased in the Peripheral Villages.

It might also be urged that the reductions noted in (1) and (2) above might have been encountered in the natural course of events, regardless of any antimalaria measures, since it has already been shown that the intensity of malaria might vary considerably from year to year and from village to village, and, during the season under consideration, the carrier anopheline was definitely less prevalent in the Central than in the Peripheral Villages.

At any rate, even admitting that the reductions were due to the anti-malaria measures, it would be difficult to evaluate the rôle of plasmoquine.

Our own judgment would be, 'not proven'.

SUMMARY AND CONCLUSIONS.

(1) A malaria survey is outlined, covering a period of 89 weeks and 2 malaria seasons, and involving 154,260 captures and identifications of 15 species of anophelines, 98,913 dissections of salivary glands, 7,154 identifications of larvæ, and five spleen and parasite surveys of 7 villages in 9½ square miles in the southern part of Birbhum District, Bengal.

(2) The region as a whole is shown to be hyperendemic, but there are wide variations in the intensity of malaria from village to village, and, in certain villages, from year to year.

(3) Benign tertian malaria is shown to be the most prevalent infection in both autumn and spring epidemics, whereas malignant tertian is next in prevalence in the autumn, and quartan is second in order of frequency in the spring.

(4) Kala-azar is shown to be present in the region surveyed and tends to complicate the spleen and fever picture in some villages more than in others.

(5) *A. philippinensis* is shown to be the chief carrier in the summer and autumn.

(6) *A. annularis* and *A. pallidus* are shown to be the only other carriers of probable importance in this region.

(7) *A. culicifacies*, *A. minimus* and *A. aconitus*, which have been shown to be carriers in other parts of India, were relatively scarce in the surveyed region and were never found infected.

(8) *A. pallidus* was the only species found to have been infected during the cold-weather months preceding the spring epidemic. During these months, *A. philippinensis* was very much reduced in numbers and was never found infected. *A. annularis* was very numerous, but was never found infected during these months.

(9) Parasite surveys indicated that gametocytes were quite frequent in the population at all times of the year and probably most frequent in December. The sporozoite rates in the carrier anophelines were found to be highest between July and October. Each of these anophelines attained its greatest density at a period of one to three months later than its highest sporozoite rate. It would therefore appear that these anophelines have a period of optimum infectivity with malaria, and that this period precedes the season of their greatest density. The optimum period of infectivity of *A. annularis* would seem to have been between July and August, for *A. philippinensis*, August and September, and for *A. pallidus*, September.

(10) *A. philippinensis* was probably equally infected during both autumn seasons of the survey, but *A. pallidus* was much more infected during the first than the second, and *A. annularis* much more during the second than the first.

(11) Comparison of larval and adult seasonal prevalences indicated that the pupal stage of *A. annularis* may have been considerably prolonged during the last three months of 1932, and that *A. barbirostris* is a non-domestic mosquito.

(12) The three carriers in the region were found to breed only in still, clean, shady, water, with considerable aquatic vegetation. Rice fields were not found to be important breeding places of any of the anophelines.

(13) *A. philippinensis* is shown definitely to prefer human to bovine habitations. It apparently leaves the houses by day and returns to them again at night, attaining its maximum density in the houses in the later hours of the night.

(14) *A. annularis* and *A. pallidus* are probably like *A. philippinensis* in regard to their preference for houses and their nocturnal habits, but they exhibit these characters to a much less marked degree.

(15) Other anophelines in the region, which are not concerned in carrying malaria, notably *A. hyrcanus* (var. *nigerrimus*), *A. vagus* and *A. subpictus*, exhibit a preference for bovine to human habitations and to be more frequently found in cowsheds and houses by day than by night. Of these three, *A. hyrcanus* (var. *nigerrimus*) exhibits the most marked preference for bovine habitations, and *A. subpictus*, the greatest frequency by day.

(16) It is suggested that there may be a connection between the preference of certain carrier anophelines for human habitations, and the qualities in these mosquitoes or their environment which make them carriers.

(17) Anophelines which can be captured in houses and cowsheds more frequently by day than by night are suspected of having other sources of nourishment besides human or bovine blood.

(18) The effect which differences in prevalence in houses and cowsheds, and by day and by night, may have on the numbers of catches of each species, when different technique as regards times and places of catching is employed, is shown in graphs.

(19) The results of a study of the effect of partial plasmoquinization in reducing the incidence of malaria in villages are shown to be inconclusive.

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OBSERVATIONS ON MALARIA IN ASSAM, WITH SPECIAL REFERENCE TO COLD WEATHER AND PRE-MONSOON ANTI-LARVAL CONTROL.

BY

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INTRODUCTION.

For many years it has been recognised that the general distribution of malaria intensity in towns, in villages and in tea estates in Assam is subject to great variation. There are non-endemic areas, and areas with varying degrees of endemicity in which the local transition of intensity from a low to a high degree may be either abrupt or gradual.

In spite of the recognition that there are varying degrees of endemicity, anti-larval measures, wherever adopted, have been put into operation on the principle that, although there are these varying degrees of endemicity, the *periods* of the transmission, and the factors responsible are the same in all cases. In adopting this principle, it has been generally recommended that anti-larval control should be started about the 15th March and continued throughout the monsoon period up to the end of November, that is, during the period when the malaria incidence was more or less intense. The factors responsible for the rise in the malaria case incidence starting in March and April, and the reasons for the differences in the monthly periods of the peaks of intensity, do not appear to have been sufficiently investigated.

The scope of this paper is to record certain observations made while controlling malaria surveys of 19 towns and tea estates in the plains of the Brahmaputra Valley of Assam. These observations have particular reference to the types of breeding places and the breeding periods of *A. minimus*, the only significant vector so far incriminated in this area, and to the direct bearing of these factors upon the varying periods and the degree of the intensity of malaria found in the survey areas. The findings show clearly that there are differences in breeding periods and corresponding differences in the monthly malaria incidence curve, and that, according to the differences in such periods of breeding, so will the periods of anti-larval control have to differ to be effective and, at the same time, economical.

PREVIOUS OBSERVATIONS IN ASSAM.

Various observers, Chalam and Young (1923), Watson (1924), Strickland (1925a), Ramsay (1929; 1930), Manson (1931) and Rice and Savage (1932), have shown that the relative proximity of a perennial stream to habitations is a factor in the determination of the absence or presence of malaria.

Observations by Chalam and Young (1923), Strickland (1925a; 1929) and Ramsay (1929; 1930) have incriminated *A. minimus* as being the significant vector species. The observations of these workers have been further confirmed by Manson (1931), Gupta, *et al.* (1932), Rice and Savage (1932), Manson and Ramsay (1932; 1933) and Rice (1935), as applied to the plains of the Brahmaputra Valley of Assam, Cachar and in certain areas of Sylhet.

From these observations, we know the important vector mosquito to be controlled is *A. minimus*, and we know its principal habitat to be moving water, be it perennial river, stream, drain, bhil, or other water of this description.

Manson and Ramsay (1932) conclude that 'perennial rivers and streams are endemic breeding grounds of *A. minimus* during the cold weather months and until the onset of the monsoon when these watercourses become silt-laden'. The same authors (1933) conclude that 'treatment of winter breeding resorts is not advocated owing to the fact that malaria is not transmitted during the period of the year when the minimum temperatures are consistently below 60°F.'. Under 'Anti-larval Work' they state, '.....it is evident that there is a high degree of infection during November, and the finding of gut infections in *A. minimus* early in April shows that anti-larval work should begin not later than the middle of March in each year'. They maintain (1932), that 'among the partially immune, infections acquired in October and November, during the highest infection period of *A. minimus*, lie latent during the cold weather months and become manifest when the physical resistance is lowered by climatic conditions during the following hot weather months'.

Manson and Ramsay thus consider that infections are acquired only between the months of April to November and that, although breeding of the vector species takes place throughout the cold weather, this breeding has no effect upon the subsequent transmission of malaria. They also attribute the normal rise in the malaria case incidence, which appears in most areas in Assam during March and April, to be the result of relapses of infections carried over from the cold weather.

Manson and Ramsay (1933), in considering the flight range of *A. minimus*, suggest that this mosquito may travel distances of over 8 miles from their winter to their summer habitats. This has yet to be confirmed. Russell and Santiago (1934) in a study of the *funestus-minimus* sub-group in the Philippines, after carefully controlled flight experiments, concluded that, 'this sub-group can fly at least one kilometre (5/8 mile)'. They give many references on the general subject of anopheline flight distances, and conclude by saying 'It seems probable from all reports that Boyd (1930) has summed up the whole question fairly as follows :—

It may be safely inferred that the influence of any production from breeding places within $\frac{1}{2}$ mile radius of any population will be felt therein; at radii of from $\frac{1}{2}$ to 1 mile the influence may be doubtful, and ordinarily at radii of more than 1 mile the influence may be expected to be nil. There are, however, occasions when it is important to ascertain with certainty whether

mosquitoes produced at these more extreme distances are actually reaching the population at a central point. The necessity for such investigations is most urgent when a breeding place exists at these limits, which has a large capacity for production without any intermediate barriers, such as high hills or dense woods, between it and the central population'.

Ramsay (1929) noted that, 'with spleen rates varying from 6.36 per cent and 76.61 per cent in two gardens barely two miles apart, with other instances in this practice of a garden with a low spleen rate less than one mile away from a garden with a high spleen rate and, above all, with groups of coolie lines on the same garden separated only by a few hundred yards where spleen rates vary from 20 per cent to over 60 per cent, it is obvious that malaria is mainly a site infection'. With his finding of *A. minimus* as the vector, he appears to have considered, at that time, the flight distance of this species to be not more than one mile.

RESEARCH FINDINGS.

Experimental work completed, but not yet reported, has shown that *A. minimus* breeding continues actively, although slowly, throughout the cold weather months, and that the larvæ found in perennial streams do not remain in a stage of hibernation but emerge as adults.

In many *A. minimus* breeding places, larvæ in all stages of development, as well as pupæ of this species, were found throughout the cold weather months. On 5th January, 1935, at 11 a.m., during the coldest period of the year, a specimen of *A. minimus* was caught in a pocket of a stream in the act of laying eggs. At this period the minimum temperatures had been below 40°F. for several days, and the maximum temperatures not above 72°F.

To ascertain whether the breeding cycle of this species was completed throughout the cold weather, an experiment was undertaken in the most northerly and coldest part of Assam under conditions as nearly natural as possible. Eggs laid on 28th January by caught gravid *A. minimus* were placed immediately in a stream pocket previously cleared of all larvæ and water insects. The stream at this point was closed in by a mosquito net with gauze bottom. The bottom of the net then was so buried as to allow the water to flow through, but to exclude the entry of any other larvæ or insects. Eggs averaged 9 days to hatch; 17 days in the larval stages and 4 days as pupæ, or a total of 30 days from egg to the adult stage, and 23 adults (14 males, 9 females) emerged from 192 eggs. The minimum temperatures averaged 54°F. and the maximum 71°F. during this period.

In breeding experiment reports by Rice (1935), it was shown that *A. minimus* females required one or more blood feeds before ovarian development was apparent. It was also shown that where this species was fed on raisins and water only, there was no ovarian development up to a period of 49 days. Among those females given blood feeds and not liberated with males, eggs developed, but when oviposited the eggs failed to hatch. On the other hand, when females fed with blood were liberated with males, they oviposited from 16 days onward, and larvæ were hatched from the eggs and successfully reared to the adult stage. The conclusions were that a blood meal is essential before mating and for the maturation of the ovaries of *A. minimus*.

These findings imply that, for specimens of *A. minimus*, which emerge during the cold weather, to be responsible for laying fertile eggs capable of developing into adults during this season, it is necessary that blood feeds be taken, and in taking blood feeds, these adults are thus capable of ingesting gametocytes. Gametocytes ingested during the coldest months, December and January, may fail to develop, or development may only be retarded and later go on to the sporozoite stage with the subsequent increases of temperature in February.

Other workers have not found oocysts or sporozoites upon dissecting *A. minimus* caught during January, February and March. During our surveys, however, we found two *A. minimus* with oocysts in January, and one with sporozoites in February, among the small number of 104 dissections, but the ages of the infected mosquitoes were not estimated.

From these researches and from our dissection findings during a cold weather, it is not considered that this subject of transmission during the cold weather has been sufficiently studied, and it is suggested that, although the numbers of *A. minimus* found during this season of the year are comparatively small, transmission may still continue.

MALARIA SURVEY METHODS.

Three types of areas were surveyed :—healthy, moderately endemic and hyper-endemic. These were done with a view to :—(a) confirming the vector species in each type of area; (b) determining the amount of malaria present by spleen and parasite indices; and (c) discovering the breeding places, and the period of breeding, of the different carrier species suspected.

For surveys made in large towns, a Sub-Assistant Surgeon, Public Health Department, who had been previously trained in malariology, was posted to each town for at least ten months. With the Sub-Assistant Surgeon was posted one or two trained collectors according to the size of the town. In smaller towns and on tea estates, frequently a collector only was posted after a preliminary survey of two to four weeks, but in all cases, either in large or small towns or on tea estates, the collectors remained on duty throughout the full year.

On being posted to a selected survey area, their first duty was to collect all relevant data for a period of the previous five years, including meteorological, mortality, total morbidity and malaria morbidity data. Their next duty was to prepare a map showing all water areas of every description, the habitations, roads, etc. After these maps were completed, for purposes of easy recording of information, they were then divided into squares representing an area of approximately $\frac{1}{4}$ square mile. From these original maps one hundred copies were duplicated, and for each month of the year a separate map was used, one for the recording of the larval findings according to breeding places, and one for adult catches according to the map square where they were caught. As the surveys progressed, periodic spleen and parasite indices were also recorded according to the map square. All water areas—still, running, shaded or otherwise—were searched regularly, so that each monthly map showed the breeding conditions and larval findings for that month, as well as the adult catches. The actual numbers of each species of larvæ and adults caught, and the results of dissection, were recorded on appropriate forms according to the breeding place, etc.

Larvæ, as collected from each separate breeding place, were sent in tubes to Shillong for identification and record. Each tube contained, with the larvæ, a slip showing the breeding number, the date of collection, and the name of the

TABLE I.
General classification of groups.

I	II	III	IV
	Group A.	Group B.	Group C.
1 Area type	Healthy	Moderately endemic.	Hyper-endemic
2 Unpolluted clear perennial flowing water, including seepages.	Absent	Present	Present
3 Unpolluted monsoon and post-monsoon clear flowing water, including seepages.	Absent	Absent	Present
4 Unpolluted perennial still clear water.	Present	Present	Present
5 Unpolluted monsoon and post-monsoon still clear water.	Present	Present	Present
6 Significant breeding habitats of <i>A. minimus</i> .	Nil	No. 2 *	No. 2 * No. 3 *
7 Occasional breeding habitats of <i>A. minimus</i> .	Nil	? No. 4 * ? No. 5 *	No. 4 * No. 5 *
8 Significant breeding period of <i>A. minimus</i> .	Nil	No. 2—Nov. to June.*	No. 2—Nov. to June.* No. 3—June to Nov.*
9 Occasional breeding period of <i>A. minimus</i> .	Nil	? No. 4 } June to ? No. 5 } Nov.*	No. 4 { June to No. 5 { Nov. but may continue longer.*
10 Approximate spleen index range.	2-10 per cent (imported cases).	20-30 per cent	45-80 per cent
11 Approximate parasite index range.	1-5 per cent (imported cases).	20-35 per cent	50-90 per cent
12 Type of malaria case-incidence curve.	Remains low and nearly flat.	Rapid rise to peak in June-July and rapid fall thereafter.	Rapid rise to peak in June-July, remaining high until Oct.-Nov.

* Numbers in Columns III and IV refer to serial number of item in Column I.

town. The larvæ were identified in Shillong by the microscopic method only, and the examiner had no means of knowing the type of water from which the specimens came. By this method the personal element of error was eliminated, and the actual numbers of each species caught were shown for each separate breeding place. The macroscopic method of identification, especially in the hands of poorly educated collectors, was not considered to be a sufficiently reliable one upon which to base recommendations for anti-larval work, and was not used.

Where a trained Sub-Assistant Surgeon was in charge of a survey, adult identifications and dissections were made and recorded by him. A certain percentage of catches were pinned for verification of his identifications. Upon dissection, all specimens found positive for sporozoites or oocysts were required to be mounted, together with the wings and legs of the infected specimens, and forwarded to Shillong for confirmation. By this method we were assured of the correctness of identification and of infection. Where a collector only was on duty, adult catches were forwarded to Shillong in Barraud's boxes for identification and dissection.

For the parasite indices, blood slides, both thick and thin films, were taken from the child and adult population, and the splenic enlargement, if present, recorded at the same time. Spleens were palpated with the patients in the standing position. The name, age, sex, caste and spleen size of each patient were recorded by serial number, and the corresponding number written on the thin-film portion of the blood preparation. These were forwarded to Shillong, where they were stained and examined by a trained staff, and the results of the examination recorded according to the different species of parasite found and to its stage of development.

Periodic visits of inspection were made to each of the survey areas by the Research Officer and the Field Supervisors. No survey was considered complete without a full year's collection of data.

SURVEY FINDINGS.

During the course of the surveys, 256,708 anopheline larvæ and 43,693 adults were identified, and 12,338 adults were dissected.

In order to determine the variations, or the absence or presence of malariogenic factors, in healthy, moderately endemic and hyper-endemic areas, surveys were completed in places falling under each of these categories. The original classifications were based upon the orthodox method of accepting the spleen and parasite rate indices as representing the weight of the malaria intensity in a given survey area.

Subsequent studies have shown, however, that certain factors are responsible for the different gradations and periods of the malaria intensity and, upon these factors, a classification is made by placing survey areas into Groups A, B and C respectively.

General characteristics of the breeding habitats and the periods of breeding of the vector species, according to the grouping suggested, and of the resulting periodicity of intensity of the malaria case-incidence curves are shown in Graphs I to IV and in Table I.

Groups A, B and C may thus be defined as follows :—

Group 'A' (Healthy Areas) are characterised (a) by the absence of any unpolluted, perennial, or monsoon-period clear, running water within one-half

to two miles of the survey areas; (b) by the absence of any significant or occasional breeding of the vector species, *A. minimus*; (c) by low spleen and parasite rates; and (d) by a malaria case-incidence curve which remains consistently low throughout all seasons of the year.

Group 'B' (Moderately Endemic Areas) are characterised (a) by the presence of unpolluted, clear perennial, running water, in which significant breeding of the vector species, *A. minimus*, continues from October or November, throughout the cold weather and pre-monsoon months, until the advent of the monsoon rains in June, when there is flushing of these water areas; (b) by the almost complete absence of unpolluted monsoon and post-monsoon clear flowing water; (c) by the occasional breeding of the vector species which may take place in unpolluted, perennial and monsoon, still clear water from June to October or November; (d) by moderate spleen and parasite rates; and (e) by a malaria case-incidence curve which, rising gradually in March, April and May, reaches its peak in June or July, and thereafter falls abruptly at first and then more slowly to a low level by October and November to remain low until March.

Group 'C' (Hyper-endemic Areas) are characterised (a) by the presence of unpolluted, clear perennial, running water in which significant breeding of the vector species, *A. minimus*, continues from October or November, throughout the cold weather and pre-monsoon months until the advent of the monsoon rains in June, when there is flushing of these water areas; (b) by the presence of monsoon and post-monsoon, unpolluted clear running water in which there is a continuation of breeding of the vector species from June to November or December; (c) by occasional breeding of the vector species which may take place in unpolluted, perennial or monsoon, still clear water at any time of the year; (d) by high spleen and parasite rates; and (e) by a malaria case-incidence curve which, rising in March, April and May, reaches its peak in June or July, remains at a high level until October or November and then falls, first abruptly, and then more slowly, to reach its low point by February.

Examples of the survey areas according to their characteristics are described briefly :—

GROUP A. EXAMPLE OF HEALTHY AREA.

TEZPUR.

The principal characteristic of Tezpur is the absence of any medium-sized or small river, stream, unpolluted drain or seepage within the town area. On the other hand, there are present practically every known condition of *still* water, such as lakes, tanks, swamps, rice-fields, borrow-pits and pools, some having clear, unpolluted, and others silty water, both shaded and unshaded, with and without grassy banks, and with and without vegetation growing up through the water. In none of these areas is the water flowing, except in the case of a few equalization drains passing through culverts. The town is situated directly upon the bank of the large Brahmaputra river, and, when its waters fall in the post-monsoon season, some of the water in low-lying portions of the town drains away. This is the only season of the year when flowing clear water is found. The nearest unpolluted, clear, flowing water is in the Mora Bhorelli stream, about $\frac{1}{2}$ mile from the western edge of the town.

Of 29,164 anopheline larvæ caught, only two were of the species *A. minimus*. These were caught in November in a clear water drain flowing to the Brahmaputra river. One-half mile outside the town, in the Mora Bhorelli stream, many *A. minimus* larvæ are found from January to June.

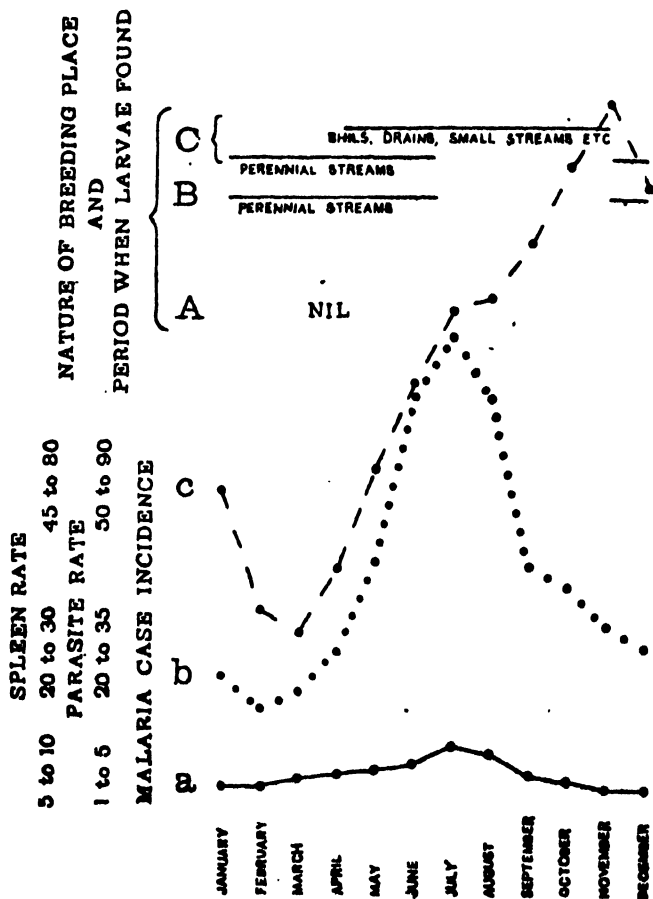
Of 7,313 anopheline adults caught, only two were of the species *A. minimus*.

The spleen and parasite rates of 1,785 and 984 children (aged from 2 to 10 years) examined were 6.3 per cent and 1.9 per cent respectively, and the highest spleen and parasite rates were recorded amongst children on the western edge of the town, nearest to the Mora Bhorelli stream. Villages within 3 to 5 miles of the town, and along this stream, have spleen rates among the children varying from 20 to 32 per cent.

The malaria case-incidence curve among the towns-people simulates that shown as *a* in Graph I.

GRAPH I.

A. minimus breeding and the related malaria case incidence.



TINSUKIA.

This town was chosen for survey by virtue of its reputation for freedom from malaria, and because it is closely surrounded by areas of endemic and hyper-endemic malaria.

Tinsukia has no river, stream, unpolluted drain or seepage within the town area. There are, however, many areas of clear, unpolluted still water such as tanks, swamps, rice-fields, borrow-pits and pools. The town is not near the Brahmaputra, or other large river. At the end of the monsoon season, water drains away through rice-fields towards streams on the north and the south of the town, but this is the only period when moving clear water is present. The nearest clear flowing stream, the Tingraijan, is approximately two miles from the southern edge of the town. Along the Tingraijan, tea estates and villages have spleen and parasite rates ranging from 25 to 50 per cent. One tea estate within five miles of Tinsukia, known to the writer, has a spleen rate of 82 per cent.

Among 5,456 anopheline larvæ and 2,434 anopheline adults caught, no specimen of the species *A. minimus* was found.

The spleen and parasite rates of 703 children (aged from 2 to 10 years) examined were 10·1 per cent and 8·4 per cent respectively. Among those examined were included 135 children who were new-comers to the town, and who had spleen and parasite rates of 36·7 per cent and 31·7 per cent respectively. Of children of permanent residents, the spleen and parasite rates were 3·7 per cent and 3 per cent respectively.

The malaria incidence curve of Tinsukia residents could not be obtained separately, but the low spleen and parasite rates suggest that it would almost certainly resemble that of *a* in Graph I.

GROUP B. EXAMPLE OF MODERATE ENDEMICITY.

NORTH LAKHIMPUR.

This town has two perennial unpolluted clear water streams, the Goraijan and Hundari, on its immediate western boundary. These streams are 8 to 12 feet broad, have sandy banks with pocket formation and grassy edges. From early June to October they are both subjected to flushing by reason of heavy monsoon rainfall. Other clear, still unpolluted water is present at all seasons in tanks, swamps, rice-fields, borrow-pits and pools. Several perennial streams, also subjected to monsoon flushing, are present within five miles of the town.

At the end of the monsoon season water flows from the low-lying rice-field areas into the two streams.

Of 19,252 anopheline larvæ caught, 963 were of the species *A. minimus*, and of these 940 were taken from the two streams between the months of December to June. One specimen of this species was found in a tank during April, and the balance (22) were taken from small seepages along the stream edges—10 in July, 3 in August, 1 in October and 8 in November.

The adult anophelines caught numbered 2,710, of which 117 were of the species *A. minimus*.

The spleen and parasite rates of 1,065 children (aged from 2 to 10 years) examined were 38·7 per cent and 23·1 per cent respectively for all areas of the town, and were noticeably higher in the closer proximity to the streams.

GRAPH II.

A. minimus larvæ: their habitat and periods when found.

	Group A		Group B												Group C.													
	Water conditions		Water conditions	Breeding periods												Water conditions	Breeding periods											
				Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sep.	Oct.	Nov.	Dec.		Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sep.	Oct.	Nov.	Dec.
1. Large Rivers, sandy banks	⊕		⊕													⊕												
2. Medium sized Rivers			+	+	+	+	+	+	+	Flushed	⊕	+				+	+	+	+	+	+	Flushed	⊕	+				
3. Small Rivers			+	+	+	+	+	+	+	Flushed	⊕	+				+	+	+	+	+	+	Flushed	⊕	+				
4. Perennial Streams			+	+	+	+	+	+	+	Flushed	+	+				+	+	+	+	+	+	Flushed	+	+				
5. Monsoon Streams			+							Flushed	⊕					+						Flushed	⊕					
6. Monsoon Streamlets, clear																+						+	+	+	+	⊕		
7. Perennial Drains, dirty water	+		+													+												
8. Perennial Drains, clear water			+	+	+	+	+	+	+	Flushed	+	+				+	+	+	+	+	+	Flushed	+	+				
9. Monsoon Drains, clear water																+						+	+	+	+	⊕		
10. Irrigation Channels																+	+	+	+	+	+	⊕	⊕	⊕	⊕	+	+	
11. Swamps & Shale, no seepages	+															+						⊕	⊕	⊕	⊕	⊕		
12. Swamps & Shale with seepages																+	⊕	⊕	⊕	+	+	+	+	+	+	+	⊕	
13. Rice Fields, no irrigation	+		+													+												
14. Rice Fields, with irrigation																+					⊕	⊕	+	+	+	+		
15. Contour Drains, dry in cold weather	+															+						⊕	⊕	⊕	⊕			
16. Seepages																+	+	+	+	+	+	+	+	+	+	+	+	
17. Lakes, no inlet	+		+													+												
18. Lakes fed from streams																+	+	+	+	+	+	⊕	⊕	⊕	⊕	+	+	
19. Tanks, rain water	+		+													+												
20. Tanks, fed from streams																+	+	+	+	+	+	⊕	⊕	⊕	⊕	+	+	
21. Pools	+		+													+						⊕	⊕	⊕	⊕			
22. Borrowpits	+		+													+						⊕	⊕	⊕	⊕			
23. Wells	+		+													+						⊕	⊕	⊕	⊕			

REFERENCES

Water conditions

Type of water present

A. minimus breeding

Larvæ sometimes found

⊕ +

+

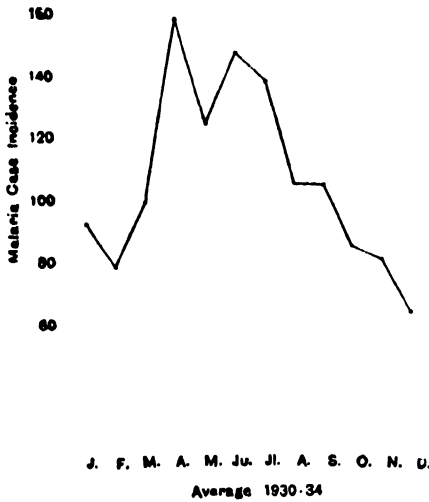
⊕

The character of the malaria case-incidence curve of those treated in the Civil Hospital, from the town and immediate surrounding villages, is shown in Graph III.

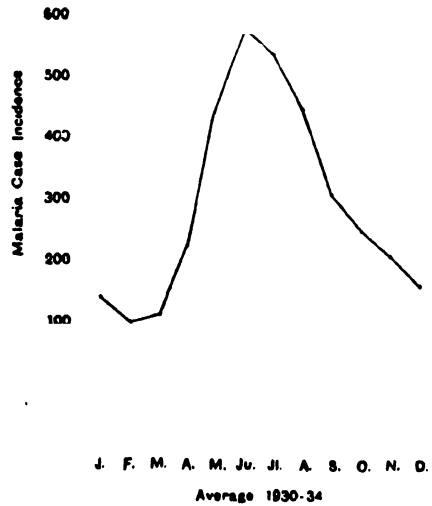
GRAPH III.

Malaria case incidence. Group B.

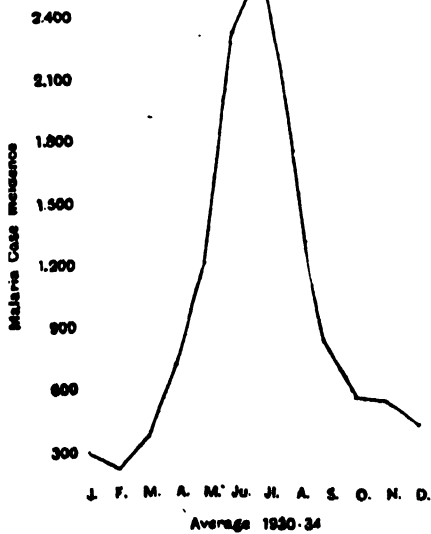
Charduar



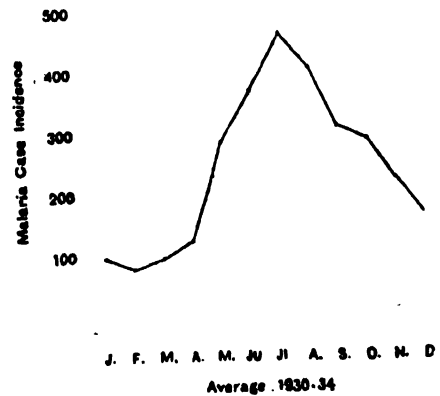
North Lakhimpur



Nowgong



Jorhat



CHARDUAR.

This frontier station, located at the foot of the Daffa Hills, has an unpolluted, clear water, perennial stream, the Mansiri, as its immediate eastern boundary. This stream has several small subsidiary streams emptying into it. In several places old dead loops of the river are found, but these have been artificially silted up so that where water collects in these loops it is still in character. Other still clear water is present in pools, borrow-pits, rice-fields and swamps.

Among 6,583 anopheline larvæ caught, 1,349 were of the species *A. minimus*. Of these, 1,270 were caught between December and July, of which 1,198 were recovered from the Mansiri river and 72 from tributaries before they dried in the cold weather. In drains and small streams, during the monsoon and post-monsoon seasons, 71 *A. minimus* larvæ were caught. Eight larvæ of this species were found in still water pools and bheels.

The adult anophelines caught numbered 2,010, of which 548 were *A. minimus*.

The spleen and parasite rates of 190 children examined were 36.2 per cent and 31.9 per cent respectively.

The character of the malaria incidence curve is shown in Graph III. This is placed in Group 'B' by virtue of the drop in incidence after July. It will be noticed, however, that there is an early rise in incidence in March, with the high peak in April.

Other examples of surveys classified under Group 'B' are Dhubri, Dimapur, Gauhati, Golaghat, Jorhat, Lokra, Nowgong, Pasighat and one tea estate.

GROUP C. EXAMPLE OF HIGH ENDEMICITY.

DOOM DOOMA.

Doom Dooma town and two estates in this district have been surveyed, and are of such similarity that they can be taken as representing one Group C area, in that the town and estates are all located on the large bheel where breeding and other conditions are the same.

This area is traversed by several small perennial rivers, all of which have tributary streams. There are also several extensive swampy bheel areas along the course of the rivers, some of which are used for rice cultivation and for cattle grazing. For the most part, however, they are vast expanses of grassy waste land made unsuitable for use by reason of flooding during the monsoon season. In the cold weather and pre-monsoon periods, the rivers are small, wandering, clear, unpolluted streams, and many of the small tributaries are dry. The bheels also tend to dry up, leaving small scattered pool formations and, in some places, seepages with small streamlets leading to the rivers. Light rains in March and April extend these seepages and streamlets.

With the monsoon rains, the rivers and tributaries are flushed and swollen to overflowing, when bheels are flooded and seepage areas increased. During this season monsoon streamlets, drains (both natural and man-made), rice-field irrigation and the swamps and bheels with clear, unpolluted and flowing water make suitable breeding places for *A. minimus*, until the post-monsoon months when the waters in the rivers and bheels gradually subside.

In this Doom Dooma area 5,226 *A. minimus* larvæ have been caught, of which 3,513 were recovered from perennial streams, irrigation channels, seepages, and perennial drains, between the months December to June. During the months July to November 1,713 larvæ of this species were recovered from monsoon streamlets, drains, swamps and bhils with seepages, rice-fields with irrigation, and small numbers from clear water borrow-pits, pools and wells.

The adult catch of *A. minimus* numbered 2,473 (representing about 25 per cent of the total anopheline catch). Dissection of 1,411 specimens showed 4.04 per cent infected with sporozoites, and 5.38 per cent with oocysts.

Spleen and parasite rates among children (aged 2 to 10 years) averaged 78.8 and 85.2 per cent respectively on the estates, and 56 and 41 per cent respectively in the town. In the latter, however, prophylactic treatment was being given to all children.

The malaria case-incidence curve for the town is shown at 'c' in Graph I; and the estates in Graph IV.

MANGALDAI.

This town is included in the Group 'C' classification, because of the secondary rise in the malaria case incidence in November, December and January. Prior to 1929, when the Mangaldai river was diverted to a new channel, leaving its old bed containing running and still clear water, this town would have been included in Group 'B'. Subsequent to the diversion of the river, malaria has increased by an average of 300 to 400 per cent annually. The secondary rise in the malaria case incidence has resulted from breeding taking place in the old bed of the Mangaldai river, when it starts to flow as a result of draining into the new bed when the level of the latter commences to fall in September and October.

Of 20,743 anopheline larvæ caught, 882 were *A. minimus*, of which 786 were caught from perennial streams between December and June. Of the remaining 96, 4 were recovered from drains during September, and 92 from the old bed of the Mangaldai river during September, October and November. Thus active breeding continues here from September to the following June. Of 2,813 adult *Anopheles* caught 220 were *A. minimus*.

The spleen and parasite rates of 478 children (aged 2 to 10 years) averaged 46.8 and 38.2 per cent respectively.

The malaria incidence curve is shown in Graph IV. Other Group 'C' areas are Sibsagar, Tangla and Nongpoh.

DISCUSSION.

The malaria incidence curves in most parts of Assam start their initial rise in March, and this initial rise has been ascribed to one or more of the following factors:

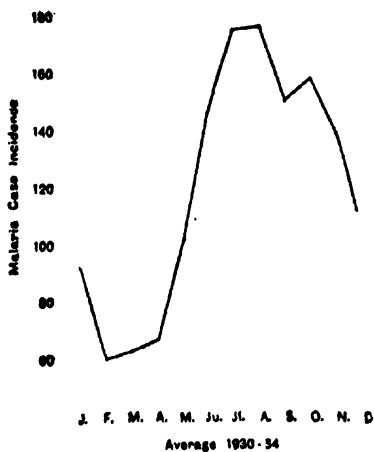
1. The residual infections in man, acquired during the late post-monsoon and early cold weather seasons, lie dormant throughout the cold weather and become manifest when the physical resistance is lowered by climatic conditions during the hot weather (this factor should not, however, affect the initial rise in March).

2. Low temperatures, by inhibiting feeding stimulus of *A. minimus*, are the limiting factors in the transmission of malaria, but with increasing temperatures the feeding stimulus becomes active and transmission commences.

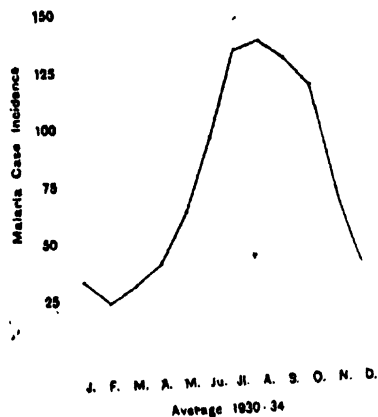
GRAPH IV.

Malaria case incidence. Group C.

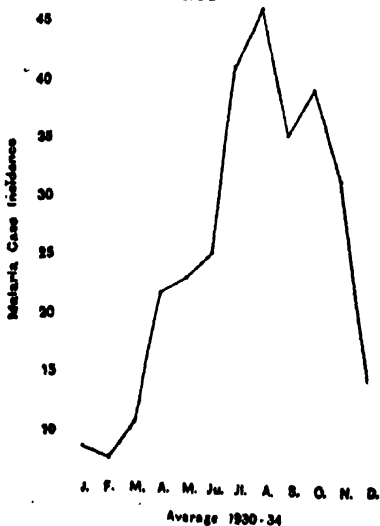
Sibsagar



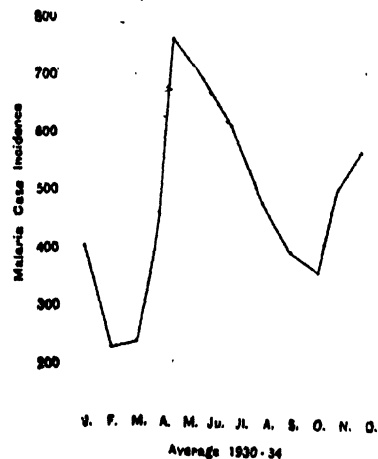
H. Tea Estate



L. Tea Estate



Mangaldai



3. The breeding of *A. minimus* is negligible during the cold weather months and only reaches a significant degree by 15th March.

4. That the sporogony cycle in the vector is retarded by temperature, and that oocyst infections acquired in the late post-monsoon and early cold weather do not reach the sporozoite stage until April.

While it is possible that these factors are responsible in a limited degree for the seed with which to start the initial malaria case-incidence rise, but, for the continuation of the subsequent rapid rise seen in both Groups B and C, in some places as early as April (Charduar, Graph III), it is possible that infections of fresh broods of *A. minimus* must also be present.

The findings described previously have shown that adult *A. minimus* can emerge during the coldest season of the year, that blood feeds may be taken by them during this period, and that the breeding cycle in the cold weather requires approximately 30 days from the egg stage to adult emergence. These adults, having emerged, must take at least one blood feed from a gametocyte carrier, the process of sporozoite development must be completed and man develop active malaria before the malaria case-incidence curve is affected. Even in the warmest weather, this cycle will take approximately 30 days to complete, therefore approximately two months must elapse between the laying of eggs and the time when the malaria case-incidence curve can be affected by these broods of the vector. With the colder weather during these early months, these cycles may even take a longer period. Thus to be responsible for the rapid rise in the malaria case incidence which is seen from April on, breeding would appear to be active in January and in February, and from then on, increasing rapidly as the temperature conditions become more suitable to breeding.

That none of these four factors operate later in the year when, during August, we see a sudden fall in the malaria incidence curves in Group B, is obvious. What then is the reason for the different transmission periods in Group B and in Group C during this portion of the year when residual and acquired infections are abundant, the feeding stimulus of *A. minimus* active, the breeding of this species not retarded by cold weather conditions, and the sporogony cycle, due to favourable weather conditions, being quickly carried on to the sporozoite stage?

When Groups A, B and C are considered separately from the standpoint of their respective differences in

- (1) the water conditions observed,
- (2) the resulting larval and adult findings,
- (3) the resulting spleen and parasite indices, and
- (4) the type of malaria case-incidence curve found,

it becomes apparent that it is not theoretical hypotheses but the actual water conditions present and suitable for the breeding of *A. minimus*, and the period at which these water conditions should be treated, which confront us as the paramount problem of malaria control by anti-larval methods in Assam.

It has been shown in *Group A areas* that no breeding of *A. minimus* takes place, and that, although breeding of this species may take place from a distance of $\frac{1}{2}$ to 2 miles, this breeding has no malariogenic effect upon the population resident within the survey area. This shows the usual maximum flight distance of *A. minimus* to be $\frac{1}{2}$ mile or less. In the absence of running streams, within such areas, no larvæ can be carried in by drifting with the current to subsequently hatch out as adults. In these areas no anti-larval activities are required.

In Group B, breeding of *A. minimus* has been shown to take place only in clear unpolluted running water, previously described in the definition of Group B areas and in Graph II, and then only in significant numbers from November to June. Considering the type of malaria case-incidence curve in these areas together with the larval findings, it is obvious that no significant breeding takes place after the onset of the monsoon and therefore anti-larval work, if carried on after flushing occurs, would be unnecessary. That breeding taking place in these areas during the cold weather months is the means of increasing the numbers of infections from those carried over the cold weather by the different factors mentioned, has been discussed. It is apparent that anti-larval activities must be carried out in these Group B areas, starting not later than early January, to stop the geometric progression in numbers of *A. minimus*, and continued until nature, by flushing the streams, has taken up the control in June. If anti-larval measures are effectively applied during this period the mathematical probability of any occasional breeding during the remainder of the year becomes diminished, and thus a nucleus for the commencement of breeding during the following season very greatly minimised. With the knowledge that the significant flight distance of the vector is not more than $\frac{1}{2}$ mile, then, if we control areas for approximately $\frac{3}{4}$ mile from the periphery of the area to be protected, we shall, by applying control measures during the period recommended, be setting up a vicious cycle directed against *A. minimus*.

The anti-larval methods to be directed against the Group B breeding places of *A. minimus* may be oiling, the application of paris green, artificial flushing (where practical without scouring), polluting of the water and the institution of dense shade. In connection with shading, this measure, first recommended to be applied against *A. minimus* by Strickland (1925b), has had considerable success because, being a permanent measure, it operates as efficiently in the cold weather and pre-monsoon seasons as it does in the monsoon season. Control measures should include the prevention of larvæ drifting into the control area by the methods recommended by Sinton and Majid (1935).

In Group C areas, the breeding activities of *A. minimus* come under the same category of suitable water conditions present, and during the same periods of breeding, as was the case in Group B, up to the time flushing occurs in June. After this period, when significant breeding stops in Group B, there is a transference of habitat to monsoon streams, drains and other suitable breeding places in Group C (see Table I), where breeding continues, and, as a consequence of this, the malaria case incidence continues high for a longer period than it does in Group B areas.

With the same features responsible, in both groups, for breeding *A. minimus* during the first half of the year, the same periods and methods of anti-larval activities are recommended, and, if these are carried out efficiently in Group C areas during the period early January until nature's flushing control is established, the mathematical probability of significant numbers of the vector being present to transfer their habitat to monsoon period clear water will be reduced. In many Group C areas, besides river, streams, drains and irrigation channels which may be controlled by oiling, applying paris green, artificial flushing, pollution or shading, the drifting-in of larvæ will have to be controlled. Seepages are often responsible breeding places, and these must be effectively oiled, paris

greened or densely shaded. During this first half of the year, anti-larval control must be efficient and must be extended to every breeding place within 1 to $1\frac{1}{2}$ miles of the periphery of the area to be controlled. If this be not done, a delayed rise in the malaria incidence curve is liable to occur, due to the infiltration of adults, thus a transference of habitat to the much extended suitable breeding areas present during the monsoon months, and consequently rapid geometric increases of the vector in these new habitats. In Group C areas, the extension of the January to June control measures to 1 to $1\frac{1}{2}$ miles is suggested, to prevent the adults which would otherwise have emerged within these limits, gradually invading the area to be protected, to subsequently start breeding in the suitable water areas present during the monsoon months, and to be responsible for a late rise in the malaria case-incidence curve. How efficient this may prove will have to be tested, for if there is still a slow infiltration with subsequent significant breeding, then control measures may have to be applied farther afield during the January to June period or, as an alternative, within the $\frac{1}{2}$ mile limit during the period June to November, whichever proves the cheaper method.

CONCLUSIONS.

The application of anti-larval activities during the period early January to mid-June (*i.e.*, until such time as nature assumes control of these breeding places of *A. minimus*), would be more economical than the present advocated period of anti-larval control (15th March to 15th November) as, during the former period, much smaller water areas would have to be controlled, because the breeding habitat of the vector would be more concentrated. Breeding then would be stopped at its lowest point in the cold weather, preventing not only the geometrical increase in the numbers of the vector, but, in its absence, preventing the propagation of gametocytes and residual infections, thus protecting the uninfected population.

The bulk of the malariogenic areas in Assam, particularly in the case of villages, come under the *Group B* classification. Often, in the case of villages, a single small stream is the responsible breeding habitat. The significance of these findings is therefore obvious, for, if we can control malaria by the application of anti-larval measures applied to the small areas of running water present in the cold weather and pre-monsoon months, it becomes an economic possibility to alleviate the plight of the bulk of villagers in Assam.

SUMMARY.

1. The breeding conditions of *A. minimus*, the most important vector of malaria in Assam, fall into three separate groups which are defined.

2. The study of the malaria problems in two of these group areas shows that breeding of *A. minimus* during the period early January to mid-June is the principal factor responsible for the increase of malaria incidence in moderately endemic and hyper-endemic areas.

3. It is suggested that anti-larval measures applied during this period of the year would be the most economical and efficient method of dealing with the malaria problem in Assam.

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THE MORPHOLOGY OF MALARIAL PARASITES.

BY

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[15th August, 1935.]

THE usual result of air drying of thin films of any kind is a flattening out of the cells as a whole and a contraction of the cellular constituents. This results in a picture which, while it may be characteristic for any particular fluid or tissue, is not a close representation of the real morphology. Such distortion of structure may to some extent be avoided by the use of vapour of osmic acid (osmium tetroxide), which fixes objects in the shapes they were in at the time of exposure to the vapour. This effect is well illustrated by fixing actively moving amœbæ in Schaudinn's fluid, with and without previous exposure to osmic acid vapour. In the former case the pseudopodia are well shown, being fixed while extruded, whereas in the latter they are withdrawn and the amœbæ are more or less rounded. It was, therefore, thought that a truer picture of an actively amœboid malaria parasite would be obtained by fixing wet blood films over osmic acid vapour previous to drying in air. On staining such films, it was found that the parasites had a characteristic appearance, differing in some respects from those seen in ordinary air-dried films. In the literature available to the author, there is no record of such a difference in the appearance of the parasites. The method of fixation is, however, not new; Sinton (1922) used it, along with other methods, for *Plasmodium tenue*. The present note relates to the simian strains of malaria—*Plasmodium knowlesi* and *Plasmodium cynomolgi*.

TECHNIQUE OF FIXATION.

The technique is very simple and differs little from the ordinary way of making blood films. Monkeys were injected with the strains of malaria, and when the infection was well established, as judged by the appearance of a large number of parasites in peripheral blood, a thin film was made on a slide in the usual way, with blood drawn from the pricked finger. However, before the film could dry, it was exposed to the vapour of osmic acid for a period of about 15 seconds by inverting the slide over the open mouth of a wide-mouthed bottle containing a 2 per cent solution of osmium tetroxide in 1 per cent chromic acid solution. At the end of this period, the thinner portions of the film, at its

periphery, would have dried, and drying of the rest of the film was allowed to take place on the bench in contact with air. At the same time as these films were made, for comparison, films were also made in the usual way, *i.e.*, they were allowed to dry in contact with air without exposure to osmic acid vapour. Both sets of films were fixed in methyl alcohol for 5 minutes, and then stained with Giemsa stain—1 drop of the concentrated stain per c.c. of buffered distilled water. The drawings which illustrate this account were made with an Abbé camera-lucida, the source of illumination being a 'Pointolite' lamp. Smears of *P. knowlesi* were all from *Silenus rhesus*, and of *P. cynomolgi* from *S. sinicus*.

COMPARATIVE MORPHOLOGY BY THE TWO METHODS OF FIXATION.

An infection, wherein the parasites were *all* in the same stage of development, would have provided an ideal condition for a comparative study of parasites by the two methods of staining, for then, films could have been made at regular intervals by both methods and their appearances compared; the fallacy that perhaps parasites of different stages were being so compared could have been obviated. Unfortunately, in every monkey so far examined, parasites have been found in different stages of development at the same time, with the result that, particularly in young ring forms, the decision as to which is the younger and which the older form becomes a matter of conjecture. However, there are striking differences in the appearance of the parasites as stained by the two methods. Without going into details, the following main differences will be noted:—

1. Osmic vapour fixation brings out more emphatically the amœboidicity of the parasites.

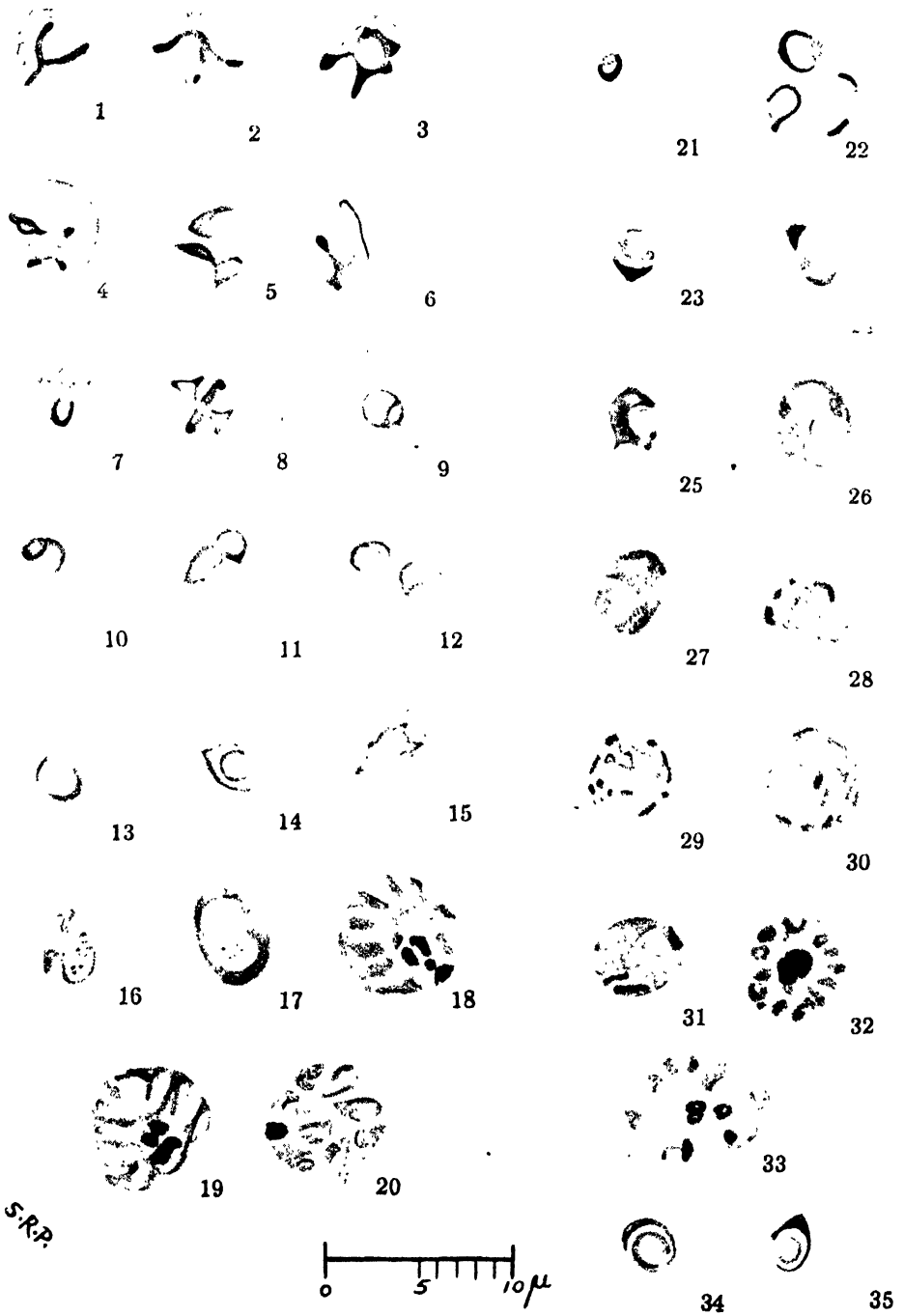
2. The chromatin mass appears to be more mobile, following actively the movements of the cytoplasm.

3. In ordinary films, the chromatin is usually seen as a homogeneous, round, red dot in young forms. Sometimes, its centre stains darkly with a lighter-stained periphery. Often there are one or more 'accessory' dots, and rarely the chromatin is crescentic (Plate IV, figs. 34 and 35), or resembles a horse-shoe with slightly bulbar ends (Plate IV, fig. 23). In osmic-vapour-fixed films, the chromatin is seen to be more spread out, covering a much larger area and staining less densely.

4. In films fixed by osmic acid, two vacuoles can be made out in many of the forms, one of which is very distinct and is surrounded by pink staining chromatin, and another which is less distinct and is surrounded by the blue staining cytoplasm. In some parasites, chiefly in very young rings, this second vacuole surrounds the chromatin mass and in others it lies to its side. Such an appearance is rarely obtained in air-dried films and a very thorough search has to be made for them. Two such forms, in air-dried films, are shown in figures 26 (plate IV) and 62 (plate V).

5. In films fixed by osmic acid, certain very peculiar forms of the parasite are seen in the case of *P. cynomolgi*. The nuclear vacuole, instead of being completely surrounded by chromatin, appears to open out into the red blood cell through a cleft in the chromatin (Plate V, figs. 46, 47, 49 and 51). Such forms have not been seen in air-dried films.

PLATE IV.



EXPLANATION OF PLATE IV.

P. knowlesi from *S. rhesus*.

Figures 1-20. From wet films fixed by osmic acid vapour.

„ 21-35. From air-dried films. Figures 34 and 35 represent parasites
seen at the periphery of a very thin film.

EXPLANATION OF PLATE V.

P. cynomolgi from *S. sinicus*.

Figures 36-51. From wet films fixed by osmic acid vapour.

„ 52-63. From air-dried films.

PLATE V.



36



37



38



52



53



39



40



41



54



55



42



43



44



56



57



45



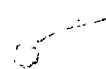
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47



58



59



48



49



60



61



50



51

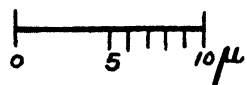


62



63

S.R.P.



6. There is little or no difference in the appearance of mature forms of the parasite by the two methods of preparing films. Very few drawings have therefore been made of such forms.

7. It will be seen that in films exposed to osmic acid vapour, the red blood corpuscles appear smaller and have a purplish colour, whereas in the air-dried films they are larger and take a light pink stain. The average diameter of an infected red cell in *P. knowlesi* infection (host, *S. rhesus*) is 7.7μ and 6.9μ respectively, in air-dried and osmic-acid-fixed films, and 8.6μ and 7.6μ respectively in *P. cynomolgi* infection (host, *S. sinicus*).

DISCUSSION.

It has been demonstrated that, when wet films of blood in malaria are exposed to osmic acid vapour and are then stained, characteristic appearances of the parasites are obtained, differing in many respects from those usually seen in ordinary films. This difference in the appearance is clearly not due to any alteration in the staining reaction brought about by the use of osmic acid vapour *as such*; for, when films which have already dried in air, are later exposed to the vapour, the parasites, on staining, differ in no respect from those in films not so exposed. The change is, therefore, due to the action of the vapour on the parasites while they are still alive. Osmic acid is known to be a strong protoplasmic poison, and the appearance of parasites in wet films exposed to it, suggests that their death has been brought about quickly, and that they have been fixed before any shrinkage or contraction has occurred; in other words, this method gives a truer picture of the parasites. It appears as if, in a film which is dried in air, death of the parasites takes place slowly, and the chromatin (which is apparently contractile) shrinks and is seen as a dense round mass. The centre which is denser than the periphery stains more deeply. Sometimes, when contraction has taken place at more than one point, the chromatin is seen at two or more points—the chromatin mass and accessory dots. Occasionally there is a thin filament joining two masses. In the same air-dried film, particularly if it has been made very thin, one might find at the periphery, which has dried quickest, parasites in which a large percentage have a crescentic or almost annular chromatin. Two such parasites from the periphery of a film are shown in figures 34 and 35 (plate IV). The edge of a thin blood film, which dries exceedingly quickly, appears to be the nearest approach to a wet film which has been exposed to the vapour of osmic acid. In this, the chromatin is usually found to be less dense and more spread out, and, particularly in young ring forms, it has the appearance of a thin veil or mantle covering a vacuole.

That there is a nuclear vacuole, as distinct from the cytoplasmic vacuole, in the malarial parasite has been recorded, *e.g.*, Sinton (1934), in describing *P. inui*, the quartan parasite of monkey malaria from ordinary air-dried films, draws special attention to the 'nuclear' vacuole and the 'protoplasmic' or 'pseudo-vacuole'. He finds that the line of demarcation between these is usually determined with ease by critical illumination in well-stained films. Such forms are seen as well in *P. cynomolgi* in ordinary air-dried films (Plate V, fig. 62), but one has to search long for them. A similar form with a suggestion of two distinct vacuoles is also shown in Plate IV, fig. 26,

in an air-dried film of *P. knowlesi* infection. With osmic-vapour-fixed films, such forms are almost the rule, and, even in very young ring forms, one can see the nuclear vacuole as distinct from the protoplasmic vacuole in both kinds of malarial infections.

Mention has already been made of certain forms of parasites (Plate V, figs. 46, 47, 49 and 51) where the nuclear vacuole appears to open out into the cytoplasm of the red cell. It is more than likely that this is due to shrinkage of the staining portion of the nuclear chromatin.

The optimum exposure to the vapour of osmic acid has not been worked out; but films exposed for longer than 15 seconds until complete drying took place over the vapour, did not show parasites in any way different from those already described, but the discoloration of the parasites was more marked.

It would be interesting to study the morphology of other species of *Plasmodia*, and in fact other blood parasites, by this method.

SUMMARY.

The morphology of the parasites of monkey malaria, *P. knowlesi* and *P. cynomolgi*, as seen in ordinary blood films and in wet films exposed, before air drying, to the vapour of osmic acid, is compared, and certain differences in their appearance are noted. It is suggested that fixation of wet films over the vapour of osmic acid gives a truer picture of the parasites.

ACKNOWLEDGMENTS.

This work was done under the direction of Lieut.-Colonel H. E. Shortt, I.M.S., the Director, when the writer was employed under the Indian Research Fund Association at the King Institute, Guindy. It is a pleasure to record my thanks to him for innumerable suggestions and advice, and also for obtaining from Lieut.-Colonel J. A. Sinton, v.c., o.B.E., I.M.S., Director, Malaria Survey of India, the two strains of monkey malaria.

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OBSERVATIONS ON THE ABSORPTION AND EXCRETION OF ATEBRIN.

BY

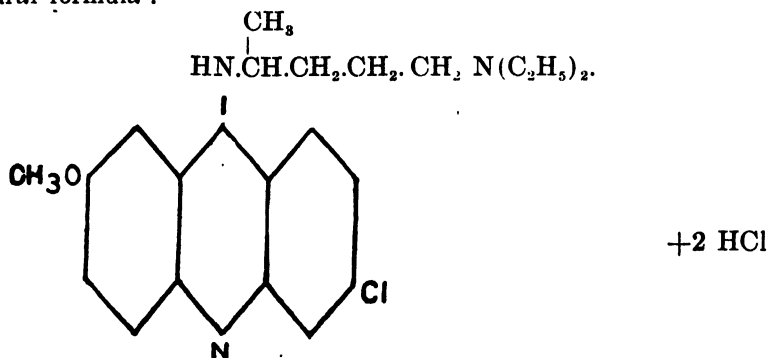
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[2nd October, 1935.]

INTRODUCTION.

WHILE considerable work has been done upon the rates of absorption and excretion of quinine, our knowledge of these processes in connection with the newer antimalarial drugs, plasmoquine and atebtrin, is much less complete. A better understanding of these is needed not only (a) because there is evidence to suggest that these drugs may tend to accumulate in the body, and thus give rise to toxic manifestations of various kinds under certain conditions, but also, (b) because the rates of absorption and excretion may be closely related to the values of these drugs in the prophylaxis, either clinical or causal, of disease. The present investigation has been made with atebtrin.

Atebtrin is the first synthetic antimalarial with a pronounced schizonticidal action on all species of human plasmodia (Kikuth, 1932; Peter, 1935). It is an acridine derivative and is said to be the dihydrochloride of 2-methoxy-6-chloro-9- α -diethyl-amino- δ -pentyl-amino-acridine, and to have the following structural formula :—



Atebtrin became available for general purposes in April 1932, and, because of the shorter duration and smaller cost of treatment (Barrowman, 1933)

besides other advantages, it is reputed to possess over quinine and plasmoquine (Green, 1934), it has been the subject of much investigation from various aspects of the problem of the treatment of malaria. However, in spite of all these efforts we have still much to learn about the action of this drug.

Amongst the other fundamentals of an ideal antimalarial drug, as enumerated by Sinton (1930), and later by Green (1934), it is of importance that such a drug should be quickly absorbed by the organism so as to start its action as a plasmocide soon after ingestion, and it should be excreted slowly so that it can serve as a prophylactic against the sporozoite injections for a reasonably long period after the administration of a therapeutic dose. The cinchona alkaloids, though absorbed rapidly, are also excreted quickly. Given by mouth more soluble salts of quinine will begin to appear in the urine within 15 to 30 minutes, and are eliminated in the greatest quantity within 3 to 12 hours (Deadrick, 1911).

The present work was undertaken to investigate the rapidity with which atebrin appears in the urine, and the duration of its excretion by the kidneys after three doses of atebrin in the day, a total of 0.3 gm.

PREVIOUS WORK ON THE EXCRETION OF ATEBRIN.

Most of the previous work on the excretion of atebrin has been of a qualitative nature. Green (1932) observed that atebrin continues to be excreted in the urine for eight or nine days after the conclusion of a seven-day course of treatment. Jarvis (1932) examined the urine of five patients after a similar course, and found that it was present for an average period of 26 days (in one instance it persisted for 37 days). Neumann and Le Doux (1931) found it present in the urine for 36 days after the last dose. Hecht (1933) records that atebrin is slowly excreted, and, even after a single dose, traces of the substance can still be demonstrated in the organism for a number of days. Tropp and Weise (1933) found that this drug is excreted in both the urine and the faeces, and state that excretion proceeds for a very long time, but the total quantity is only a fraction of the amount administered. Massa (1933) demonstrated the excretion of atebrin up to two months after the termination of the treatment.

EXPERIMENTAL METHODS.

Observations were made both on human beings and on monkeys.

A. OBSERVATIONS ON HUMAN BEINGS.

Four volunteers of healthy physique were invited from the laboratory staff. They were divided into two groups. Observations on one group were started in September 1934, and on the other in April 1935. They were given three doses of dihydrochloride of atebrin (Bayer's tablets) three times a day. The first dose was given after breakfast followed by the other two, each after an interval of three hours to the volunteers of the first group, and with one-hour intervals to those of the second group. Each tablet contains 0.1 gm. of atebrin, thus making a total dosage of 0.3 gm. in the day. The drug was given orally in the same way as it is given to patients in the hospitals, so that parallel conditions between the clinical and the experimental procedures are obtained. The only difference between the two being that, whereas in the full

course of treatment for malaria the drug administration is continued for five days, in our experiments the drug was given for one day only.

The volunteers were made to empty their bladders immediately before the first dose of atebirin (0.1 gm.) was administered. Their urine was then collected every half hour for the first six hours, and examined qualitatively to determine the time of the first appearance of the drug excreted by this route. The total urine passed in each 24 hours was also collected, and the amount of its atebirin content estimated by the method of Wats and Ghosh (1934). This was continued daily until traces of the drug could no longer be detected in the urine.

Wats and Ghosh claim that atebirin can be determined quantitatively when mixed in urine, up to a dilution of 1 in 1,000,000, but the results of a large number of observations in the present research appear to indicate that the quantitative accuracy of the method is impaired if the dilution of atebirin naturally excreted in the urine is more than 1 in 200,000. Beyond this range the presence of atebirin is indicated by the green fluorescence, seen through the column of the amyl alcohol extract with a converging beam of sunlight projected by means of a convex lens. Considerable difficulty has been experienced in being certain of the presence of atebirin in minute traces in the urine taken immediately after the ingestion of the drug, and also during the last few days when the excretion is very small. The greenish-blue fluorescence exhibited by the urobilin, normally present in the urine, makes it difficult at first to differentiate from the green fluorescence of atebirin. But after some experience, when the eye is trained, it is easy to detect atebirin qualitatively in minute dilutions.

B. OBSERVATIONS ON MONKEYS.

Two male normal *Silenus rhesus* monkeys weighing 4,500 and 5,000 gm. were selected. The first monkey was given a single intramuscular injection of 50 mg. of the dihydrochloride of atebirin dissolved in water, whereas the other was given daily an intramuscular injection of 50 mg. on each of five consecutive days. Both of these monkeys were kept in 'metabolism cages', and the urine was drained into a clean bottle containing a little of chloroform to prevent bacterial contamination. Twentyfour-hour samples of urine were collected; the total daily quantity was then measured, and the amount of atebirin estimated by the above method. No attempt was made in the case of these monkeys to detect the first appearance of atebirin in urine, because the only way this would have been possible was to catheterise the animals. This procedure was, however, found very unsatisfactory, since the animal will, out of fright, pass urine when an attempt is made to catch it prior to catheterisation. Moreover the total collection of urine for 24 hours also could not be taken as an absolutely correct measure of the kidney excretion, because there is no surety that the animal has emptied its bladder when the collected urine is removed after each 24-hour period.

RESULTS OF EXPERIMENTS.

NORMAL HUMAN BEINGS.

(i) RATE OF ABSORPTION.

The rapidity of absorption of a drug into the general circulation, as indicated by its appearance in urine, is an important attribute of the possible efficacy of an antimalarial drug, and has been estimated by the length of time

taken by the drug to appear in urine after the administration of the primary dose. In one set of experiments the first dose of 0.1 gm. of atebrin was given to two persons at 10-20 a.m., followed by two similar doses after an interval of three hours. Urine was collected every half hour for the first six hours. The second batch of persons was given the same total amount of atebrin but the intervals between the doses were one hour.

Table I shows that whereas in the case of the first two men the atebrin was detected in the urine after 90 to 120 minutes, in the other two cases it was found after 60 to 90 minutes.

TABLE I.

Rate of appearance of atebrin in the urine of human subjects after three spaced doses of 0.1 gm. each.

Time in minutes after administration of primary dose.	ATEBRIN GIVEN AT INTERVALS OF 3 HOURS.		ATEBRIN GIVEN AT INTERVALS OF 1 HOUR.	
	1	2	3	4
30	—	—	—	—
60	±	—	±	+
90	+	±	+	+
120	+	+	+	+
150	+	+	+	+
180	+	+	+	+

In a later set of experiments (*see* Table II) in connection with the effect of food in the stomach on the absorption and excretion of atebrin (Kehar, 1935), where a single dose of 0.3 gm. of the drug was given, it was observed that, in one case out of nine, there was a doubtful indication of the presence of atebrin within the first 15 minutes; in 6 other cases it was found present after the thirty-minute interval; in another case after a fortyfive-minute interval and in the remaining case a minute trace was detectable after 60 minutes and an appreciable amount after 75 minutes.

TABLE II.

Rate of appearance of atebrin in the urine after a single large dose (0.3 gm.).

Number of cases.	TIME IN MINUTES.					
	15	30	45	60	75	90
1	—	+	+	+	+	+
2	±	+	+	+	+	+
3	—	+	+	+	+	+
4	—	+	+	+	+	+
5	—	+	+	+	+	+
6	—	+	+	+	+	+
7	—	—	—	±	+	+
8	—	—	+	+	+	+
9	—	+	+	+	+	+

The above observations clearly indicate that the dihydrochloride of atebtrin is absorbed by the organism very soon after oral administration, and that excretion in the urine commences rapidly.

(ii) RATE OF EXCRETION.

It has been observed that, about six hours after the administration of the last of the three tablets of atebtrin, the concentration in the urine arrived within quantitatively measurable limits. Such estimations were then carried out daily till the concentration of the excreted atebtrin became insignificant, and the amyl alcohol extract failed to give any indication of the presence of the characteristic fluorescence.

Tables IV and V show a number of interesting observations. It will be noted that atebtrin is excreted in measurable quantities (*i.e.*, 1 in 200,000) for 16 to 28 days, but qualitatively its presence can be detected as long as 55 to 69 days. It will also be seen that it was possible to show that the kidney excreted from about 49 to 69 per cent of the dosage of atebtrin during 16 to 28 days after its administration. Although the excretion continued after this time, it was not possible to make any accurate estimation of the quantity. The amount of atebtrin excreted during the first five days after administration is shown below in Table III.

TABLE III.

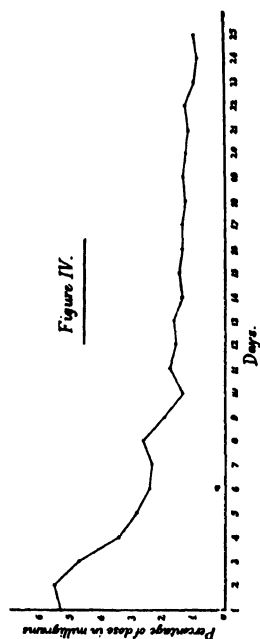
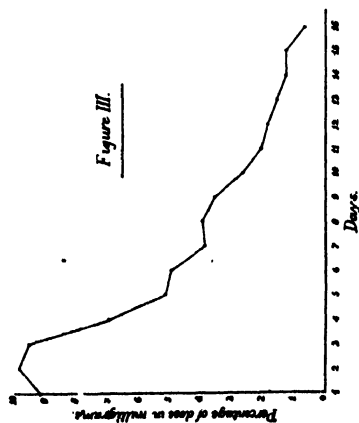
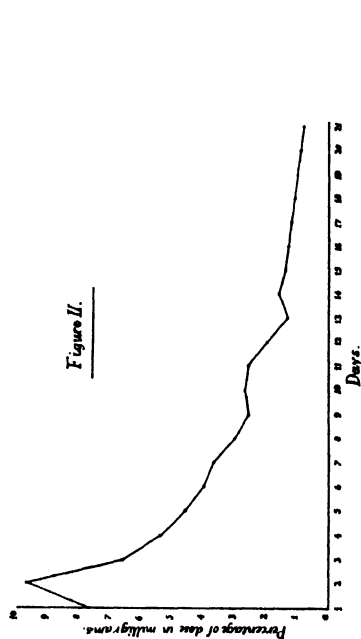
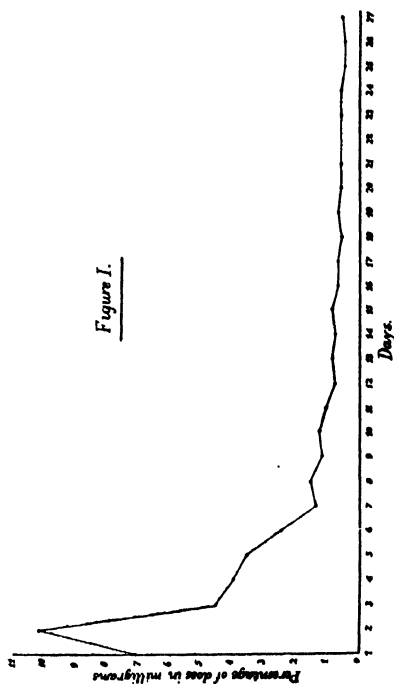
Rate of excretion of atebtrin in the urine during the first five days.

Number of cases.	NUMBER OF DAYS.				
	I	II	III	IV	V
A.	7.1 mg.	10.2 mg.	4.6 mg.	4.0 mg.	3.6 mg.
B.	7.7 "	9.7 "	6.6 "	5.4 "	4.6 "
C.	9.2 "	9.9 "	9.6 "	7.0 "	5.2 "
D.	5.4 "	5.6 "	4.8 "	3.5 "	2.9 "
Total.	29.4 "	35.4 "	25.6 "	19.9 "	16.3 "
DAILY AVERAGE PER CASE.	7.3 "	8.8 "	6.4 "	5.0 "	4.1 "

Taking Tables IV and V into consideration separately, it will be observed that, in the case of persons A and B to whom atebtrin was given at intervals of three hours, the greatest amount of excretion was on the second day of administration, showing an increase of 3 to 4 mg. compared to the first day. But when the interval between the three doses was reduced to one hour (persons C and D), the increased excretion on the second day was only about a milligram. When, however, all the three tablets are given in a single dose (Kehar, 1935), the total excretion of atebtrin during the first 24 hours following administration, is greater during that day than on any of the succeeding days.

GRAPHS I TO IV.

Figures I to IV showing the percentage of dose excreted daily.



Furthermore, it is obvious from a perusal of the tables that atebrin is very slowly excreted, and that the amount excreted decreases gradually day by day till it falls to below 1 mg. per cent of the administered dose. This takes about 16 to 28 days, and apparently depends upon the total amount of urine excreted by the individual. The greater the amount of urine the quicker appears to be the rate of excretion. It may be that in such cases the system is flushed more quickly and thoroughly compared to others in whose cases the kidney excretion is less. It will also be noted that in cases B and C, whose urinary output was larger, the rate and amounts of atebrin excreted were greater than in cases A and B, who passed less urine. These differences are more clearly shown in Graphs I, II, III and IV.

TABLE IV.

Experiment started 26th September, 1934.

1st dose 0.1 gm. atebrin at 10-20 a.m.
 2nd dose " " " 1-20 p.m.
 3rd dose " " " 4-20 p.m.

Date.	Number of days.	AMOUNT OF ATEBRIN PRESENT IN THE URINE OF THE PREVIOUS 24 HOURS.	
		A.	B.
27th September, 1934	1	10.71 mg.	11.68 mg.
28th " "	2	15.34 "	14.62 "
29th " "	3	6.95 "	10.01 "
30th " "	4	8.14 "	8.21 "
1st October, 1934	5	4.56 "	6.92 "
2nd " "	6	3.78 "	6.10 "
3rd " "	7	2.20 "	5.57 "
4th " "	8	2.81 "	4.61 "
5th " "	9	1.91 "	4.00 "
6th " "	10	2.07 "	4.11 "
7th " "	11	1.68 "	4.02 "
8th " "	12	1.25 "	3.10 "
9th " "	13	1.40 "	2.60 "
10th " "	14	1.24 "	2.45 "
11th " "	15	1.37 "	2.20 "
12th " "	16	1.19 "	2.00 "
13th " "	17	1.11 "	1.80 "
14th " "	18	1.00 "	1.75 "
15th " "	19	1.02 "	1.49 "
16th " "	20	0.95 "	1.38 "
17th " "	21	0.92 "	1.19 "
18th " "	22	0.94 "	+
19th " "	23	0.98 "	+
20th " "	24	0.95 "	+
21st " "	25	0.79 "	+
22nd " "	26	0.82 "	+
23rd " "	27	0.91 "	+
24th " "	28	.. "	+
		±	
		up to 65 days	up to 60 days
TOTAL IN MG. 	74.99	99.81

TABLE V.

Experiment started 27th April, 1935.

1st dose 0.1 gm. atebrin at 9-40 a.m.
 2nd dose " " " 10-40 a.m.
 3rd dose " " " 11-40 a.m.

Date.	Number of days.	AMOUNT OF ATEBRIN PRESENT IN THE URINE OF THE PREVIOUS 24 HOURS.	
		C.	D.
28th April, 1935	1	13.81 mg.	8.12 mg.
29th " "	2	14.90 "	8.98 "
30th " "	3	14.45 "	7.25 "
1st May, 1935	4	10.81 "	5.31 "
2nd " "	5	7.92 "	4.38 "
3rd " "	6	7.59 "	3.85 "
4th " "	7	5.95 "	3.69 "
5th " "	8	6.00 "	4.16 "
6th " "	9	5.44 "	3.10 "
7th " "	10	4.10 "	2.17 "
8th " "	11	3.25 "	2.78 "
9th " "	12	2.92 "	2.42 "
10th " "	13	2.52 "	2.58 "
11th " "	14	2.05 "	2.17 "
12th " "	15	2.00 "	2.35 "
13th " "	16	1.06 "	2.12 "
14th " "	17	+	2.10 "
15th " "	18	+	2.00 "
16th " "	19	+	2.21 "
17th " "	20	+	2.02 "
18th " "	21	+	1.85 "
19th " "	22	+	1.95 "
20th " "	23	+	1.58 "
21st " "	24	+	1.44 "
22nd " "	25	+	1.61 "
23rd " "	26	+	1.10 "
24th " "	27	+	1.25 "
25th " "	28	+	1.00 "
			±
		up to 55 days	up to 69 days
TOTAL IN MG.	104.77	85.54

THE RATE OF EXCRETION OF ATEBRIN IN MONKEYS.

The information obtained from observations on monkeys could not be taken to be of the same practical value as that from human beings because of the possible fallacies mentioned above. It is, however, interesting to note from Table VI that, in the case of monkey No. 236, the greatest amount of excretion after one intramuscular injection was during the first 24 hours. The concentration then gradually fell off until, after 25 days, it could not be measured quantitatively. The presence of atebrin continued, however, to be detected by the qualitative method up to 51 days. In the case of monkey No. 235, the excretion was greatest during the first few days after the injections, and then

dropped off slowly. Observations in connection with the duration of excretion could not be continued as this monkey died of pneumonia after 24 days.

These observations, in a measure, confirm the findings recorded on human beings about the rapid absorption and slow excretion of atebtrin, and do not support the views of Blaze and Simeon (1935) in that the maximum excretion of atebtrin occurs only after three days.

TABLE VI.

Rate of excretion of atebtrin in monkeys.

Experiment started on 4th October, 1934.

Date.	Number of days.	NUMBER OF MONKEYS.	
		235	236
5th October, 1934	1	7.05 mg.	2.19 mg.
6th " "	2	5.70 "	1.95 "
7th " "	3	4.54 "	1.49 "
8th " "	4	4.61 "	0.97 "
9th " "	5	4.44 "	0.89 "
10th " "	6	7.92 "	0.99 "
11th " "	7	5.15 "	0.90 "
12th " "	8	1.80 "	0.52 "
13th " "	9	1.29 "	0.24 "
14th " "	10	1.40 "	0.77 "
15th " "	11	0.57 "	0.40 "
16th " "	12	0.61 "	0.28 "
17th " "	13	..	0.42 "
18th " "	14	0.60 "	0.83 "
19th " "	15	0.51 "	0.61 "
20th " "	16	1.00 "	0.60 "
21st " "	17	0.44 "	0.35 "
22nd " "	18	..	0.29 "
23rd " "	19	0.34 "	0.40 "
24th " "	20	0.36 "	0.06 "
25th " "	21	0.66 "	0.64 "
26th " "	22	0.43 "	0.64 "
27th " "	23	0.50 "	0.64 "
28th " "	24	0.51 "	0.64 "
29th " "	..	Died of pneumonia	0.64 "
30th " "	+
31st " "	+
		Up to 51 days.	

DISCUSSION OF RESULTS.

The points that strike one are that atebtrin given orally begins to appear in urine after about 15 minutes, and continues to do so from 54 to 69 days. The greater the amount of urine passed the quicker is the excretion of atebtrin. These observations on the prolonged excretion of this drug are in conformity with the qualitative findings of Green (1932), Jarvis (1932), Neumann and Le Doux (1931) and Massa (1933).

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It has also been shown that, in cases where 0.1 gm. of atebrin was given at intervals of three hours, the greatest excretion was on the second day. If, however, the time interval between the doses is reduced to one hour, the excretion on the day of administration is increased almost proportionately. When, 0.3 gm. of atebrin is given in one single dose, the greatest excretion is during the first 24 hours, and then gradually falls off day by day.

Blaze and Simeon (1935) consider that absorption and excretion of atebrin during the first three days is extremely small. They also state that the clinical symptoms of malaria are not affected by atebrin therapy during the first three days, and that only the last 6 to 7 tablets of a 15-tablet course of atebrin are actually therapeutically utilised. They consider that the first 9 tablets are only necessary to saturate the liver to such an extent that it will allow the subsequent doses to leak into the general blood stream. These observations are not in agreement with our experimental observations, as shown by the tables and the graphs. The Editor of the *Indian Medical Gazette* (1935) remarked that this statement of Blaze and Simeon has been 'contradicted in practically every paper that has been published on the clinical action of atebrin'. The clinical observations of Blaze and Simeon are not in keeping with those recorded by Napier and Das Gupta (1932), Chopra, Das Gupta and Sen (1933), Chopra and Das Gupta (1933) and Chopra and Sen (1934), who report that, even in heavy infections, the parasites disappear on the second or the third day after the administration of atebrin, and that a mean dosage of 0.525 to 0.7 gm. was required to bring down the temperature. Such clinical results can only be obtained if the concentration of the drug in the general blood circulation has a parasitocidal action during this period, which eventually implies greater absorption and thereby increased excretion during the first two or three days.

Our observations (Graphs I to IV) show that, though the excretion of atebrin in the urine commences very soon after administration, there is a sharp rise on the second day compared to the previous or the succeeding days. But this can satisfactorily be explained on the assumption that, since atebrin dissolved in water forms a colloidal solution, the mucous membrane of the intestinal tract will adsorb a portion of the drug, so that in the beginning of the course, at least, a certain portion of the dose will be adsorbed by the different tissues, but as soon as no more is adsorbed, the excess, helped by the surface forces, will enter into the general blood circulation. This view is supported by the observation that when atebrin was given in doses of 0.1 gm. at intervals of three hours, the peak of the graph is sharp; but if the interval is reduced this peak is much less so (Graphs III and IV). And when a larger dose is given on a single occasion (Kehar, 1935) the excretion on the first day is more than the succeeding days.

ATEBRIN A SLOW EXCRETING ANTIMALARIAL DRUG.

The evidence obtained by the observations of other workers, and that afforded by our experimental findings, gives an ample proof that atebrin is excreted by the body very slowly. The exact mechanism of excretion is not clear.

Hecht (1933), however, advances an attractive theory, and says that, 'a large portion of atebrin taken by mouth is retained in the upper intestine, liver and bile, and circulates from the intestine to the liver and is excreted with

the bile back into the duodenum from where it is again reabsorbed into the liver. It is believed to circulate in this way before reaching the peripheral blood in any large amount'. In the absence of any convincing experimental evidence, it is difficult to accept this presumption, especially when our observations indicate that atebirin appears in the urine as early as 15 minutes after the administration of a single dose. 'Even after using quinine clinically for centuries, we still have no exact knowledge regarding the action of quinine on the malarial parasite and the cellular responses; our ignorance is, if anything, more complete regarding the action of atebirin' (Editor, *I. M. G.*, 1935).

SLOW EXCRETION AND THE PROPHYLACTIC ACTION OF ATEBRIN.

Atebrin is not considered to be either a direct symptomatic or a causal prophylactic (Green, 1934; Schulemann, 1935). But a drug may become, in effect, an efficient causal prophylactic by acting with such energy on the asexual forms evolving from the sporozoites that the development of clinical malaria is prevented. Schulemann (1935) states that atebirin does so. When given to general paralytics for six days atebirin prevented infection with *P. falciparum* entirely and, in the case of *P. vivax*, the incubation period was prolonged for several months. Kikuth and Giovannola (1933) found that the incubation period of canaries treated prophylactically with atebirin was substantially prolonged, compared to that of control animals or those protected with quinine or plasmoquine. Muhlen and Fischer (1932) asserted that atebirin was prophylactically of value because of its slow rate of excretion from the body. Soesilo, Gilbert and Bagindo (1933) applied atebirin prophylactically in practice and state that it possesses a certain preventive action.

It is possible that the protection afforded by atebirin in either prolonging the period of incubation, or checking the onset of adverse conditions of the disease, is primarily due to the therapeutic effect caused by its prolonged retention in the system. The quantity of atebirin left in the body after the first few days is enough to combat the unlimited multiplication of the parasites and to restrict the disease. But after about two weeks when the amount of retained atebirin is comparatively small, it is not sufficient to prevent the continued development of any parasites which have survived.

SUMMARY AND CONCLUSIONS.

1. If 0.1 gm. of atebirin is given orally three times a day at intervals of three hours, it appears in urine after 75 to 90 minutes, but if the intervals are reduced to one hour it takes about 60 minutes.

2. Because of the rapid absorption of atebirin after oral administration, it seems of little advantage to resort to intramuscular or intravenous routes unless in exceptional circumstances. It is, however, suggested that in cases where the onset of the disease has assumed a severe form, it may be advisable to administer 0.3 gm. in one dose on the first day of treatment to check the adverse symptoms.

3. Atebrin may continue to be excreted from the body up to 69 days after the administration of a total of 0.3 gm. atebirin at intervals in a single dose. The duration of the drug in the urine appears to be influenced by the amount of urine excreted.

4. It is assumed that, since atebrin is an acridine dye, its slow excretion may be due to its retention in the body tissues, either in a complex chemical or colloidal combination, and when the concentration in the circulation fluid decreases, the adsorbed dye diffuses out slowly and is finally excreted by the kidneys.

5. Fifty to seventy per cent of the administered dose of atebrin, at least, is excreted by the kidneys.

6. The protection afforded by atebrin in either prolonging the incubation period or checking the onset of adverse conditions of the disease, is assumed to be due to its prolonged retention in the body tissues.

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THE INFLUENCE OF FOOD IN THE STOMACH ON THE ABSORPTION AND EXCRETION OF ATEBRIN.

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THE rapid absorption and elimination of an antimalarial drug is considered by most authorities to be of great practical importance. It is also suggested that the useful part of the dose is that which goes into the general blood circulation. If these suggestions be correct, for the best therapeutic result, therefore, we require to introduce into the blood stream a sufficient amount of the drug in the shortest possible time to bring about either the destruction of the parasites, or make the environments unfavourable for their unchecked development.

Our present knowledge of the therapeutics of atebtrin is still very incomplete. More information seems needed about the best method by, and the best time at, which to administer this drug. The present investigation is an attempt to determine those conditions under which the drug should be administered so as to obtain the maximum absorption.

EXPERIMENTAL METHODS.

Some of the healthy members of the staff were selected as volunteers, the present author being one of them. The methods of procedure and estimation were the same as those mentioned in the previous paper (Kehar, 1935). The only departures from these were that 0.3 gm. of the dihydrochloride of atebtrin were given in a single dose; that urine was collected every 15 minutes for the first two hours to detect qualitatively the time of the first appearance of the drug excreted by the kidneys; and, since we were interested in finding out the maximum concentration arrived in the system immediately after the administration of the drug, the total amount of atebtrin excreted during the first three days only was estimated.

While this work on the dihydrochloride of atebtrin was in progress, the makers introduced into the market the methyl sulphonie acid salt of the same base, under the name 'Atebrin musonat'. Of the latter drug 0.125 gm. is

equivalent in atebtrin content to 0.1 gm. of the dihydrochloride of atebtrin. The manufacturers claim this salt to be more soluble. It was, therefore, considered advisable to make a comparison between the rates of absorption and excretion of these two salts when given by the oral route. Thus nine persons were given 0.3 gm. of the dihydrochloride of atebtrin, and three 0.375 gm. of atebtrin musonat, each dose with about 200 to 300 ml. of water. The experiments were planned as follows :—

- | | | |
|-----|---------------------------------|-----------------------------------|
| 1. | Atebrin dihydrochloride 0.3 gm. | given on an empty stomach. |
| 2. | " | " " " " with breakfast. |
| 3. | " | " " " " one hour after breakfast. |
| 4. | " | " " " " 1½ hours after breakfast. |
| 5. | " | " " " " 2 hours after breakfast. |
| 6. | " | " " " " 2½ hours after breakfast. |
| 7. | " | " " " " 3 hours after breakfast. |
| 8. | Atebrin musonat 0.375 gm. | " with breakfast. |
| 9. | " | " " 2½ hours after breakfast. |
| 10. | " | " " on an empty stomach. |

The breakfast, in one case (No. 5, Table I) where atebtrin was given one hour after meal, consisted of an omelet of 2 eggs, 2 slices of toasts, a cup of milk, and two ounces of grapes; in another case, No. 6, where atebtrin was given 1½ hours after meal, the meal consisted of an omelet of one egg, roast meat, 'dhal mung'* and 3 pancakes of kneaded wheat flour (chapaties). Amongst the laboratory staff (Nos. 1 to 4, and 7 to 9, Table I), however, it consisted of the usual full morning meal, comprising of 2 to 4 buttered or unbuttered 'chapaties' with some dhal*. In the first case, the components of the meal may be taken as well-balanced dietetically, in the second slightly rich in proteins, and that of the laboratory staff had a high carbohydrate content.

RESULTS OF EXPERIMENTS.

A. OBSERVATIONS ON THE DIHYDROCHLORIDE OF ATEBRIN.

(i) RAPIDITY OF ABSORPTION.

It has been pointed out before (Kehar, 1935) that the rapidity with which a drug appears in the urine is considered to be an indication of its rate of absorption in the general blood circulation. This has been estimated by the length of time taken for the drug to become detectable in urine after the administration of the dose.

Table I shows that in the case of one person, who was given atebtrin upon an empty stomach, there was a doubtful indication of the presence of atebtrin in the urine within the first 15 minutes. However, in the case of 5 persons to whom atebtrin was given either with or up to 2 hours after breakfast, it was found after 30 minutes in urine in the case of 3 persons, and after 45 minutes in the other; only in one instance was the excretion delayed, giving a doubtful indication of its presence after 60 minutes and evidence of appreciable amount after 75 minutes. In two persons who were given atebtrin 2½ to 3 hours after breakfast, the excretion of the drug was first detected after 30 minutes.

* Boiled lentils flavoured with spices.

TABLE I.
Showing the rate of appearance of atebtrin in the urine.

Mode of administration.	Number of cases.	TIME IN MINUTES.					
		15	30	45	60	75	90
1 hour before breakfast ..	1	—	+	+	+	+	+
	2	—	+	+	+	+	+
with " ..	3	—	+	+	+	+	+
	4	—	—	+	+	+	+
1 hour after " ..	5	—	+	+	+	+	+
1½ hours " ..	6	—	—	—	—	+	+
2 " " " ..	7	—	+	+	+	+	+
2½ " " " ..	8	—	+	+	+	+	+
3 " " " ..	9	—	+	+	+	+	+

(ii) RATE OF EXCRETION DURING THE FIRST THREE DAYS.

Table II indicates that when atebtrin is administered, either upon an empty stomach or 2½ hours after a meal, the percentage of the administered dose excreted during the first 24 hours is greater than when the same amount is given with or within two hours of the meal. After the administration of 0.3 gm. of the dihydrochloride of atebtrin, upon an empty stomach or 2½ hours after meal, the amount excreted by the kidneys during the first 24 hours reached 9.1 to 9.6 mg. per cent. On the other hand, the same amount administered during or within 2 hours of the meal, was followed by an excretion of 8.3 to 8.9 mg. per cent during the same period. The total quantity excreted during three days after administration of 0.3 gm. upon an empty stomach or 2½ hours after meal, was 19.4 to 19.7 mg. per cent; whereas during the same period following 0.3 gm. at meal time or up to 2 hours after meal, the quantity excreted was from 18.6 to 19.3 mg. per cent.

TABLE II.
Showing the percentage of the dose excreted during the first three days after administration.

Number of days.	EMPTY STOMACH.		WITH BREAKFAST.		AFTER BREAKFAST.				
					1 hr.	1½ hrs.	2 hrs.	2½ hrs.	3 hrs.
	Case No.								
	1	2	3	4	5	6	7	8	9
I	96	95	83	83	89	86	89	91	94
II	64	65	66	66	62	78	64	58	67
III	36	36	38	37	39	29	39	48	33
TOTAL ..	196	196	187	186	190	193	192	197	194

Table III also shows that with the administration of 0.3 gm. of atebtrin on an empty stomach or 2½ hours after a meal, the total excretion in the urine was 13.9 to 14.5 mg. during the first 24 hours, as compared to 12.3 to 13.4 mg. after a dose given with or within 2 hours of the meal. The total quantity excreted during three days was 29.2 to 29.9 mg. in the former, and 28.1 to 29.1 mg. in the latter case.

TABLE III.

Showing the total excretion of atebtrin in mg. in urine during the first three days after administration.

Number of days.	EMPTY STOMACH.		WITH BREAKFAST.		AFTER BREAKFAST.				
					1 hr.	1½ hrs.	2 hrs.	2½ hrs.	3 hrs.
	Case No.								
	1	2	3	4	5	6	7	8	9
I	Mg. 14.5	Mg. 14.2	Mg. 12.4	Mg. 12.3	Mg. 13.4	Mg. 12.9	Mg. 13.4	Mg. 13.9	Mg. 14.1
II	9.7	9.8	9.9	10.0	9.3	11.8	9.7	8.8	10.1
III	5.4	5.5	5.8	5.6	5.9	4.4	5.9	7.2	5.0
TOTAL ..	29.6	29.5	28.1	27.9	28.6	29.1	29.0	29.9	29.2
DAILY AVERAGE.	9.9	9.8	9.4	9.3	9.5	9.7	9.6	9.9	9.7

B. OBSERVATIONS ON 'ATEBRIN MUSONAT' (ATEBRIN METHYL SULPHONATE).

During the study of the absorption and excretion of 'atebrin' it was determined that, under the conditions of the experiment, the time when this drug is more quickly absorbed and excreted by the system is either after its administration upon an empty stomach, or 2½ hours after a meal. It was, therefore, considered advisable to study the absorption and excretion of 'atebrin musonat', a more soluble salt of the same base under parallel conditions. Atebrin musonat was, therefore, given to 3 persons with about 200 to 300 ml. of water. One of them received 0.375 gm. on an empty stomach, the other with meals, and the third 2½ hours after meals. It will be seen from Table IV that in the case of the first person atebtrin appeared in the urine within the first 15 minutes; in the second after 45 minutes and in the third after 30 minutes.

TABLE IV.

Showing the time taken by the drug to appear in urine.

Mode of administration.	TIME IN MINUTES.					
	15	30	45	60	75	90
One hour before breakfast ..	+	+	+	+	+	+
With breakfast ..	—	—	+	+	+	+
2½ hours after breakfast ..	—	+	+	+	+	+

In addition, it is seen from the data in Table V that when atebtrin musonate is given upon an empty stomach, the excretion in the urine during the first 24 hours is 14.8 mg., compared with 13.9 mg., and 14.4 mg. when given with a meal and 2½ hours later respectively. The total excretions for the first three days after administration of 0.375 gm. of atebtrin musonate on an empty stomach, with meals and 2½ hours after meals, were 33.8, 31.7, and 33.1 mg. respectively. This table also shows the percentages of the administered dose excreted during the first 24 hours and a total of three days.

TABLE V.

Showing the total excretion and the percentage of the administered dose during the first three days.

Number of days.	EMPTY STOMACH.		WITH BREAKFAST.		2½ HOURS AFTER BREAKFAST.	
	Excretion in mg. during the previous 24 hours.	Percentage of the administered dose.	Excretion in mg. during the previous 24 hours.	Percentage of the administered dose.	Excretion in mg. during the previous 24 hours.	Percentage of the administered dose.
I	14.8	9.8	13.9	9.3	14.4	9.6
II	10.8	7.2	11.4	7.6	10.3	6.8
III	8.2	5.5	6.4	4.3	8.4	5.6
TOTAL.	33.8	22.5	31.7	21.2	33.1	22.0

C. COMPARISON OF THE RATE OF ABSORPTION AND EXCRETION OF THE DIHYDROCHLORIDE OF ATEBRIN AND METHYL SULPHONATE OF ATEBRIN.

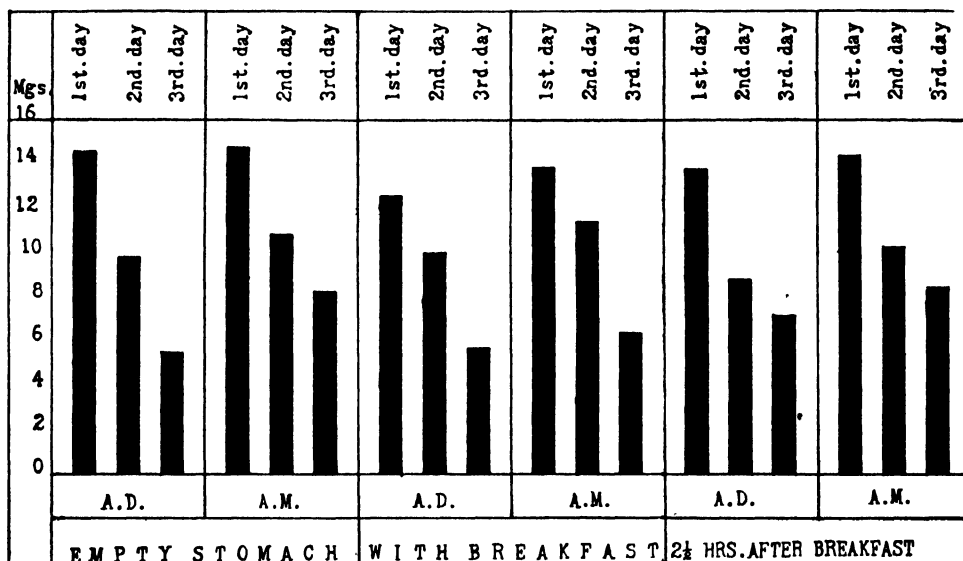
It is of interest to note from Tables I and IV that the rate with which both atebtrin dihydrochloride and atebtrin musonate appear in the urine after the administration of a single dose, is almost the same in both the cases. The total amount of atebtrin musonate, however, excreted during the first 24 hours and also during the three days following the dose, is relatively greater than is found under identical conditions following the administration of atebtrin dihydrochloride. These observations are more clearly shown in Fig. 1.

DISCUSSION OF RESULTS.

From a perusal of Tables I and IV it is clear that when the dihydrochloride of atebtrin and atebtrin musonate are given by the oral route, either on an empty stomach or 2½ hours after a meal, they take about 15 to 30 minutes to appear in the urine, but when the same dose is given with or 2 hours after a meal, it takes about 30 to 45 minutes to become detectable in the urine. Furthermore, Tables II, III and V indicate that the total excretion during the first 24 hours and during the three days following the administration of the drug, is comparatively greater when administered on an empty or a partly empty stomach than on a full stomach.

FIG. 1.

Showing the excretion during the first three days when atebtrin dihydrochloride and atebtrin methyl sulphonate are administered before, with and 2½ hours after a meal.



A.D. and A.M. represent atebtrin dihydrochloride and atebtrin methyl sulphonate respectively.

It is well known that little or no absorption of ingested material takes place in the stomach. Howell (1925) states that 'absorption does not take place readily in the stomach—certainly nothing like so easily as in the intestine'. He also remarked that 'water when taken alone is practically not absorbed at all in the stomach'. Von Mering's classical experiments showed that 'as soon as water is introduced into the (empty) stomach it begins to pass into the intestine, being forced out into a series of spurts by the contractions of the stomach. Within a comparatively short time practically all the water can be recovered in this way, none or very little having been absorbed in the stomach'.

There is much evidence to suggest that little of the allied drug quinine is absorbed from the stomach. It is highly probable that when atebtrin is given with water on an empty stomach, it soon reaches the duodenum and the small intestine, helped by the contractions of the gastric musculature, and that it is then rapidly absorbed by the system. Furthermore, when the drug is administered 2½ to 3 hours after a meal, most of the food is then leaving or has left the stomach, and the atebtrin solution also does not remain long before being pushed into the duodenum and the intestine along with the food.

When, however, atebtrin is administered with a meal it is possible that it forms an adsorption compound with the protein and starch of the food, and the delay in the initial excretion of the drug may be due to the conversion of the colloidal into the diffusible forms, or probably due to delayed passage into the absorptive portion of the gut.

In the absence of further or more extensive experimental evidence, it is difficult to say whether a particular type of diet exerts any influence on the

absorption of atebtrin. In one instance, however, where the drug appeared in the urine 75 minutes after administration, the subject had taken eggs and roast meat, whereas most of the other persons had a carbohydrate rich diet. It may be that the unusual delay in the appearance of atebtrin in the urine is caused by the influence of a protein-rich diet.

SUMMARY AND CONCLUSIONS.

1. Both the dihydrochloride of atebtrin and atebtrin musonat are quickly absorbed by the system, when administered by mouth.

2. When 0.3 gm. of the dihydrochloride of atebtrin or 0.375 gm. of atebtrin musonat are given by the oral route, either on an empty stomach or $2\frac{1}{2}$ to 3 hours after meal, the excretion of the drug in the urine can be detected within about 15 to 30 minutes. But if the drug is administered with meal or up to two hours after the meal, it takes about 30 to 45 minutes and in certain cases about 60 minutes to become evident in the urine.

3. If the dihydrochloride or atebtrin musonat are administered on an empty stomach or $2\frac{1}{2}$ hours after a meal, the excretion during the first 24 hours after administration, and, during the three following days, is comparatively greater than when given with meals.

4. Atebrin musonat after oral administration takes about the same time to appear in the urine as does the dihydrochloride of atebtrin, but the total excretion during the first three days after the administration of a dose is greater in the former than in the latter case.

5. Protein-rich diet may, possibly, exert a retarding influence on the absorption of atebtrin to a certain extent.

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WHAT MALARIA COSTS INDIA, NATIONALLY, SOCIALLY AND ECONOMICALLY.—(Contd.)*

BY

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F. ARE ANTI-MALARIAL OPERATIONS PAYING PROPOSITIONS?*

G. GENERAL SUMMARY AND CONCLUSIONS.*

H. REFERENCES.*

C. THE EFFECTS OF MALARIA UPON THE HEALTH, VITALITY, AND PHYSICAL DEVELOPMENT OF THE PEOPLE.

'The mortality from malaria in India is a mere trifle compared with the ravage "fever" commits in sapping the strength and vigour of the people' (Florence Nightingale—'Life and Death in India').

'The introduction and spread of malaria in Greece is stated by Ronald Ross, and with strong reasons, to have been largely responsible for the progressive physical degeneration of one of the strongest races of the earth' (Howard, 1909).

'Malarial fever is perhaps the most important of human diseases. Though it is not often directly fatal, its wide prevalence in almost all warm climates produces in the aggregate an enormous amount of sickness and mortality' (Ross, 1911).

'From statistics we find that as a broad general rule in malarious countries about one-third of the total population suffer from attacks every year, and also that about one-third

*The remaining sections of this article are being prepared for publication in a later number of the *Records of the Malaria Survey of India*.

of the admissions to hospital and attendances at dispensaries are due to malaria'. . . . 'The malady complicates all other diseases in the tropics in a way which renders them more difficult to treat'. . . . 'Thus the total bill of annual mortality and sickness which King Malaria presents to the human race is something enormous' (Ross, 1914).

'In the number of deaths caused either directly or indirectly the sickness and suffering, the lowered vitality of those afflicted, malaria is without rival' (Leathers, 1918).

'Diseases such as cholera and plague are terrible in their visitations; the majority of those who are infected die, a few recover, and in a few years the effects of the outbreak will have disappeared and leave behind no permanent damage to the population. It is very different in the case of malaria; the children of the fever-stricken areas are in a continual state of ill health and those who live grow to an enervated manhood. And the decay is persistent and accumulative through generations' (Nicholls, 1921).

'The same authority (Carter) points out that the gravest injury from malaria is not these sudden increases, spectacular as they are, but its continuous and increasing prevalence in communities, sapping their strength and energy' (Fuchs, 1922).

'It is malaria which is the main cause of the insalubrity of India, and, so long as it continues prevalent, widespread and severe, so long will it sap the vitality of the people'. . . . 'It is the main cause of the high sickness rate, especially among the rural population' (Hehir, 1927).

'Infection with the malaria parasite plays a very important part in lowering standards of health and physique. In every plantation area, whether in North or South India, malaria causes most of the sickness and is chiefly responsible for the existence of "unhealthy gardens"' (Royal Commission on Labour in India, 1931).

'By destroying the blood, weakening physical resistance, and ruining mental energy and moral determination, malaria has a disintegrating effect on the race and paralyses and annihilates human energy and resources by rendering the earth, our mother, inhospitable and pestilential' (Celli, 1933).

Various workers have made estimates varying between 100 and 200 millions, as the number of persons who suffer from malaria in India each year. Christophers (1926) states that, at a low estimate, the amount of sickness is 50 to 100 times the one or two million deaths, which are usually considered to occur each year from malaria. Chopra (1933) estimates that there are 100 million individuals suffering from malaria in India, who are untreated.

In the earlier parts of this paper it has been estimated that, at least, two million people die each year in British India from the direct and indirect effects of malarial infection. The loss caused by these deaths is a relatively small matter as compared with the damage done by the sickness produced by this disease. Malaria is undoubtedly the greatest cause of ill health, suffering and misery in the tropics, and is almost certainly the disease which is the greatest single cause of morbidity in the world. It prevents the proper physical and mental development of the people, and, by lowering their vitality, is probably one of the most important factors in diminishing the expectation of life among the people of malarious areas.

(I) MORBIDITY CAUSED DIRECTLY BY MALARIA.

Fletcher (1932) states that this disease affects one-third of the human race. Ross (1911) thought that probably one-fourth to one-half of the total attendances in hospitals and dispensaries in malarious countries are due to this disease. It is said by Cowan (1929) that Professor Muller of Cologne has estimated that out of the world's population, 800,000,000 persons, almost half the total, suffer from this disease. The Malaria Commission of the League of Nations gives the figure at 650,000,000, or about one-third of the people in the world.

'The great mass of India, with its population of 319 millions, nearly one-fifth of the population of the whole world, equal to the population of Africa and South America combined, and probably twice that of all other population in the tropical and sub-tropical zone, excluding China, comes under conditions which may be described as a varying but often moderate (malarial) endemicity' (Christophers, 1930).

The incidence of malaria in India must be well above the mean for the whole world, so that if one takes the average estimate of one-third, mentioned above, we should not be assuming an excessive proportion. This figure would mean that at least 90 million persons are suffering from malaria in British India, and 120 million in the Indian Empire.

It is very difficult to obtain any precise information as to the morbidity which is directly due to this disease in India. The Public Health Commissioner with the Government of India reports that during 1931 over 11,000,000 cases labelled as 'malaria', or 15.4 per cent of the total attendances, were treated at official hospitals and dispensaries in India. These numbers do not show the extent to which the population of this country is infected, although they indicate the great prevalence of the disease and the havoc which it must play. These figures merely represent the patients who voluntarily seek medical aid at public institutions, and do not take into account those who are untreated, or who are treated by private practitioners of either the Eastern or Western systems of Medicine. In the rural areas of India, where malaria is most prevalent, private practitioners of medicine are usually conspicuous by their absence, and the Government dispensaries are the only means of obtaining proper treatment in such areas. Although these institutions each provide for an area with an average radius of 10 miles, transport difficulties are so great that the patients who attend such official institutions are often drawn from a comparatively small distance around each. The patients only represent that fraction of the total sufferers which is in a position to avail itself of hospital advice and treatment. Many of the sick are unable to come or have no facilities for carriage over distances of several miles to hospital, often over very poor roads or paths. In addition, the routine work of such institutions does not permit the medical man to leave his base for long journeys, except in very rare instances. The result is that probably the vast majority of sufferers from malaria in India recover or die without any proper treatment.

Bentley (1925) has published some statistics with regard to the incidence of malaria in different parts of Bengal. The 'fever index'* in the Western division of that province in 1920 was 55 per cent, in the Central division 45 per cent, in the Northern division 30 per cent, and in the Eastern division 12 per cent. From these figures he has calculated that 1,811,739 individuals, out of a population of 45,483,077, attended Government institutions for malaria in the year under review. This means that an average of 4 per cent of the total population attended hospitals for malarial treatment in Bengal, i.e., 6 per cent, 5.7 per cent, 4 per cent, and 2 per cent respectively in each of the different divisions. He estimates that a total of about 30 million individuals, or about 66 per cent of the population of Bengal, suffer from malaria each year (*vide* Table I). If this rate were applicable to British India as a whole, at the

* The figures for 1911 are given in Table I. Bentley uses the term 'fever index' to denote the proportion of cases of 'fever' to the total number of cases treated in Government institutions.

present time it would mean that about 180 million people suffer from malaria annually, or 234 million in the whole of the Indian Empire.

If 1 per cent of the population were afflicted with enteric fever, it would be considered a most severe outbreak, yet a rate of 40 to 60 per cent from malaria does not appear to have attracted attention at all commensurate with its importance.

Bentley (1913b) carried out a series of investigations in two areas of the Dacca district of Bengal. One of these had an average spleen rate of 0·8 per cent, and the other 25 per cent. In the comparatively healthy area, the travelling dispensaries were called upon to treat about 12 per cent of the population for sickness of all kinds, and about 1·1 per cent for 'fever' and 'spleen'. On the other hand, in the more malarious area, the proportions were 34·5 per cent and 15 per cent respectively. These figures indicate the marked increase in the general morbidity in an area where malaria is prevalent.

The morbidity due to malaria has been studied in Bombay City by Bentley (1911b) and by Covell (1928). The former author found that 50 per cent of the admissions into the Police Hospital were due to 'fever', and of these 75 per cent were diagnosed malaria by microscopical examination of the blood. From these figures he calculated that there were probably 375,000 cases of malaria among the population of Bombay at that time. Covell (1928) using similar figures calculated that there were about one million cases of the disease each year among a population of 1,175,914 persons in this city. A spleen census made by the latter worker of 27,647 children in the schools of the city shows an average spleen rate of only 8·1 per cent (variations between 0 and 62·2 per cent). This investigation was made, however, when malaria was increasingly prevalent in the city.

In the Report of the Royal Commission on Agriculture in India it is stated that one-fourth of the 45,000,000 people in the United Provinces get two attacks of malaria each year, and only 1 per cent of these ever receive proper treatment.

Under special conditions, the morbidity rates from malaria may be very much higher. Thus Christophers (1912), in the convict settlement in the Andaman Islands, found that 50 to 70 per cent of the admissions to hospital were due to malaria.

During the malarial epidemic of 1908, Christophers (1911a) reports that in Amritsar almost the entire population of 160,000 persons were prostrated, and the ordinary business of the city was interrupted. Murray (1909) states that the attendances at the hospitals and dispensaries of the United Provinces for the five years 1903—1907 were 2,994,641, or an average of 600,000 per annum. During the epidemic of 1908, the number was 1,369,583, or about twice normal. Mackenzie (1923) points out the terrible effects of epidemic malaria upon the population of South Russia. He states that the survivors were so weak that they could neither plough, collect food, nor look after their few animals.

Clyde (1931), in speaking of the condition of 'tropical aggregation of labour' during the construction of the Sarda Canal in the Terai of the United Provinces, mentions that sickness from malaria was so prevalent, that at one time the contractors refused to proceed with the work, and one after another left. At one period, 96 out of every 100 individuals of the imported labour

force were down with fever. Senior-White (1928) also stresses the high malarial morbidity among workers during railway construction work in certain parts of India.

Some workers consider that, at a maximum, only about 1/10th of the persons suffering from malaria in India ever receive treatment. If this be so, then the sufferers in this country must equal, at least, 10 times the number of persons who attend hospital for malaria each year. These attendances at official institutions in 1931 are given at about 11 million, so on this basis there were 110 million cases of malaria in British India in that year.

As remarked by Maxcy (1923), 'it seems pertinent, however, to emphasize the fact that the real importance of malaria as a burden upon the public is not reflected in the mortality returns, however useful these may be in indicating the intensity of its geographical distribution'. Such mortality figures may be useful, however, in making an estimation of the amount of malarial sickness which occurs in India each year.

The direct case mortality from malaria usually lies between 0.5 and 2 per cent. The former figure is probably low for India, as it is based upon the results of treated cases, while in this country the majority of such patients receive no treatment. The latter figure is probably high, for it has been compiled mainly from the results of cases admitted into hospital, and these are usually on an average the more severe ones. A case mortality of 1 per cent among untreated or partially treated patients would not appear to be an excessive figure upon which to base any calculations.

If our previous estimate of at least one million deaths per annum, directly due to malaria in British India, be reasonably accurate, then, from the case mortality, it may be calculated that the morbidity due to this disease must lie between 50 and 200 millions per annum. From the data at our disposal, it appears almost certain that, at least, 100,000,000 individuals suffer each year in India from malaria, and possibly twice this number may be affected.

Macdonald (1931a) estimates from a series of careful observations that splenic examination only detects from 40 to 50 per cent of individuals infected with malaria. The Malaria Commission of the League of Nations (1930) gives the spleen rates of 236 villages in Bengal, of 242 in Madras and 93 in Mysore, and more than half these villages show spleen rates over 25 per cent. In Mysore the general average was found by Sweet among 8,143 school children to be 22.3 per cent. Gill (1928) reports that, as the result of spleen examinations carried out twice annually in 29 different districts in the Punjab between 1914 and 1923, the average spleen rate in that province was found to be almost 12 per cent. In taking this figure it must be remembered that the Punjab, apart from fulminant epidemics, is not, generally speaking, a very malarious province as compared with India as a whole. It must also be noted that the rates given by Gill and Sweet were determined on school children. The latter are well known to have a lower rate than the average child population taken at random, because the children of the lower social classes, and where economic stress is present, less frequently attend schools, and in the latter type of population malaria is more common (*vide* 'Economic Stress', p. 252). Megaw (1933) thinks that a splenic index of 14.3 per cent would give a fair average of the prevalence of malaria in India, although he considers that a more comprehensive survey is much to be desired.

If we consider these recorded spleen rates in conjunction with the figures reported by Macdonald (1931a), it appears probable that at least 30 per cent of the population, or 81 millions in British India, are infected with malaria.

In recent years, although the rate has declined markedly as the result of anti-malarial measures, about one-fifth of the British troops in India are *admitted to hospital* each year for malaria (Mackenzie, 1929b). As many of these men (i) are in the hills during the worst portion of the malaria season, (ii) receive treatment and so are less liable to relapse, and (iii) are protected by anti-mosquito operations to a much greater extent than are ordinary individuals, there is no reason to suppose that they are more exposed to infection than is the rural population of this country. If a similar ratio of infection exists among the rest of the community, there must be at least 70 million cases of malaria in India each year.

CONCLUSIONS.

From the estimates which have been made by various methods it appears very probable that *at least* 100 million individuals* suffer from malaria each year in India, and that not more than 1/10th of these attend official institutions for treatment.

(II) MORBIDITY CAUSED INDIRECTLY BY MALARIA.

The indirect effects of malaria in increasing the mortality in malarious countries have already been discussed, and the difficulties in forming any precise opinion of the extent of these effects have been pointed out.

As has been remarked by Watson (1924) 'without going into details, I would observe that the presence of malaria leads to a general weakening of the resistance of the people to the organisms, which live in, on or about man. In ordinary health, the human body can resist the entrance of such invaders; when weakened by malaria it cannot, and the person falls ill with some other disease not apparently malaria, *e.g.*, diarrhoea and dysentery from bowel organisms; abscesses from bacteria which have passed in generally from the skin or mouth. The removal of malaria from a population is invariably followed by a fall in the amount of disease from other causes as remarkable as that of the fall of malaria'. This fall in morbidity following successful anti-malarial operations has also been commented upon by many workers in different countries.

Bentley (1908) states that it is chiefly from the sufferers from malaria that the bulk of the sick in the tea gardens of the Bengal Duars are drawn, whatever might be the immediate cause of the illness. In the tea gardens of Assam, Rice and Savage (1932) estimate that 25 per cent of the balance of sickness, not directly due to malaria, is primarily brought about through the lowered resistance produced by this disease. Macdonald and Chowdhury (1931) state that, in other tea gardens in the same province, cases of malaria with abnormal symptoms are often entered under the diagnosis of the symptom which predominates. The figures for sickness due to 'other causes than

* This estimate is considered by the Health Organisation, League of Nations (1932) to be a moderate one.

malaria' show a seasonal curve very similar to that of malaria, though often the former curve reaches its height slightly later in the year than the malaria one. This is thought to be explained by the greater frequency of abnormal cases of malaria and of complicating diseases at the end of the seasonal epidemic than at the commencement. In one garden these authors calculated that the number of such cases which should have been attributed to malaria was equal to 31 per cent of those directly attributed to this disease, and this, be it noted, in a treated population.

Manson (1931), from a study of another group of tea gardens in the same area, infers that 'malaria exercises a very decided effect in determining the rate of mortality from all causes. It seems probable that the state of physical ill health induced by malaria is the starting point for such intercurrent diseases as pneumonia, phthisis, and dysentery'. Williams (1928) thinks the vast majority of the so-called dysenteries on malarious tea estates in Assam are malarial in origin, and that anæmia is largely due to this disease. He considers that, under these conditions, 'dysentery and anæmia are the best indices of malaria'. Manson (1931) also believes that a lot of the anæmia, usually attributed to hookworm, may be due to malarial infections and a similar opinion has been expressed by Murphy (1928). Christy (1935) also notes that persistent and pernicious malaria lowers the body resistance to infection giving rise to other forms of disease.

Lal and Shah (1933) remark that the lowered recuperative power of the people in the water-logged areas of the Punjab is due to poverty and repeated malarial infection. In the malarious parts of the United States, Rose (1919) considers that 70 per cent or more of all sickness disability is due to this disease.

While no exact estimate of the sickness indirectly due to malaria can be made, it is evident that it is a very serious factor in lowering the health of the people. If one accepts the estimates given above, and the estimates of the morbidity directly caused by malaria (*vide supra*), the additional indirect morbidity due to this disease must lie, at least, between 25 and 75 million cases annually.

(III) THE EFFECTS OF MALARIA UPON THE PHYSICAL DEVELOPMENT OF THE POPULATION.

Malaria is a disease which may affect almost every system of the human body, and so must have a powerful effect upon its physical development. As pointed out by Rose (1919), apart from its mortality, 'more serious still are its effects in retarding the development of individuals and communities. Like hookworm, the malaria plasmodium is an anæmia-producing parasite. It saps the life of its victims by destroying their blood. The disease is more prevalent among children under 15 years of age, and therefore preys upon the vitality of the race during the important period of physical and mental growth'.

De Brun (1910) has written of the blighting effects of malaria upon the growth of children. Conti (1910) also notes its detrimental action upon the physical development of children in Sardinia. Infantilism, cachexia and other affections resulting from malaria have been reported in many different countries.

As pointed out in an Editorial in the *United States Public Health Reports* (1919), 'the prevalence of malaria is most injurious to children, and affecting them, as it does, at the time when they should be getting their growth and education, it gives them a permanent handicap in life'.

A special study of the effects of this disease upon school children has been made by Leipoldt, Cleaver and Elias (1921) in the Transvaal. They report that, as a result of chronic malaria, the bodily strength of the children is diminished by two-fifths, the heart is affected and the blood pressure remains below normal. These workers describe anæmia, emaciation and general debility as the most conspicuous signs of chronic malaria in children.

Children suffering from the chronic form of this disease, before they develop an immunity, are bodily weak and incapable of prolonged exertion, either physical or mental. The miserable state of the children so afflicted in the Terai of the United Provinces has been graphically described by Phillips (1925).

In Italy and Hungary during the Great War, an extraordinary low percentage of the young men from malarious areas was found to be physically fit for military duty. The findings were similar in India. Bogoyavlensky (1928), in discussing the relationship between endemic malaria and the physical condition of the population, points out that, while in the less malarious mountain areas of Azerbaijan, 28·7 per cent* of recruits were rejected, in the malarious riverine zone this rose to 75·4 per cent.

It is clear from the evidence available that malaria has a markedly deleterious influence upon the physical development of both children and adults, in populations residing in localities where they are seriously afflicted with the manifestations of this disease.

(IV) THE EFFECTS OF MALARIA UPON THE EXPECTATION OF LIFE.

'Mr. Bruce Barton says "Every once in a while somebody rises up to criticise modern parents for devoting too much time to their children's physical being (as compared with their spiritual and educational). Says such a critic. . . . To lay so much emphasis on the physical is paganism. Our righteous ancestors disregarded their bodies and paid attention to their souls. This is all true, and it might be added that by neglecting their bodies in the interests of their souls, they beat us to heaven by an average of twenty years"' (Stanley, 1932).

The effect of malaria upon the vitality of the people of a country may be considered from another point of view, that of the 'expectation of life'.

Catrin (quoted by Anderson, 1927) indicates that in two neighbouring countries, one malarious, the other not, there is a striking contrast from the point of view of race feebleness, mortality and average longevity. Kelsch and Kiener (1880) and Celli (1904) noted that the average duration of life is shorter in malarious localities. A similar statement about the shortened expectation of life in India is made by Hendley (1918) and by Hehir (1927), while Balfour (1928) remarks that 'in British India, a land where hygiene has as yet made little progress, it is only 23 years', as compared with an expectation of life of 44 years in England.

* Bogoyavlensky in an abstract sent to the *Tropical Diseases Bulletin* gives this figure as 87 per cent.

A statistical enquiry into the effects of malaria in certain districts of the Bombay Presidency was made by Hutchinson (1910). He states that the census figures seem to show that the malarial areas are unfavourable to longevity. In the Kanara district, where there are many malarious areas, there were only 41 persons over 60 years of age in every 1,000 persons of the population. In areas like Bijapur where there is little malaria the proportion was 55, while in areas such as Belgaum and Dharwar, in which the malarial conditions are intermediate in severity, the figures were 51 and 48 respectively.

The action of disease upon the expectation of life among the population of India has been well summarised by Christophers (1924) in his Presidential Address at the Fifth Indian Science Congress :—

‘Now all men must die, but it is to be hoped that each will have a run for his money, so to speak, and live to a reasonable age, say 50 years. Let us see how many of the 360,000,000 of India enjoy this privilege. During the first year of life the expectation of life as given in the census is for males about 23 years. At the age of 5 it is 35 years, the highest expectation at any age. At 20 it is 27 years, and at 50 about 14 years. The expectation of life in females is not very different. How do these figures compare with those of European countries? The total death rate in Great Britain in 1911—1915 was 13·8 per mille’ (*cf.* India with about 25 per mille). ‘The expectation of life in the first year of life for males was about 44 years, and this expectation rose at the age of 3 to about 54 years, the highest point reached. At 20 it was 41 years and at 50, 19 years. We may say then that disease costs every one of the 360 million persons living in the Indian Empire on the average a loss of 44 *minus* 23, or 21 years’ expectation of life in the first year of life and 54 *minus* 35 or 19 years at the ages of 3 to 5 when the dangers of early infancy have already been passed. Every young adult of 20 stands to lose 14 years’ expectation of life and every man of 50, 5 years. It is true at the age of 50 the difference does not sound very great, but whilst in England 530 out of every 1,000 persons born reach this age, in India only 186 have done so, so that for every man of 50 in the Indian population who expects to live 14 years there should have been, had it not been for additional liability to disease, no less than three men each expecting to live 19 years’. ‘An increased death rate then is not merely a figure in a book, it is the first penalty India pays to disease, *viz.*, a decreased expectation of life to all the living’.

In a Resolution passed at the All-India Medical Research Workers’ Conference in 1924, it was pointed out that ‘the percentage of infants born in India who reach wage-earning age is about 50 per cent, whereas it is quite possible to raise this percentage to 80 or 90 per cent’. Megaw (1933) also notes that the span of life in India is less than half what it might be. This lowered expectation of life is also commented upon in the Report of the Royal Commission on Agriculture in India (1928).

The excessive mortality, direct and indirect, resulting from malaria must have a marked action in lowering the expectation of life in India, for such mortality occurs at all ages. That its fatal action is most marked in children must make it a great factor in preventing very many of these from reaching a wage-earning age. Apart from any direct action of the disease in limiting the length of life of the individual, the lowered vitality which it produces must make a very large number of the afflicted persons fall victims to intercurrent

diseases, from the effects of which, and not from the immediate result of malaria, they die. This enhanced indirect mortality is especially marked among the infants born of malarious mothers (*vide ante* pp. 258—262).

There would appear to be little doubt, therefore, that malaria, both by its direct and indirect actions, is a very important factor in lowering the expectation of life in India at all ages, but more especially among infants and children.

(V) CONCLUSIONS.

From the data available, there is very considerable evidence to show that, at least, 100,000,000 individuals suffer yearly from malaria in British India alone, and that this is probably a moderate estimate. In addition to these, there is an indirect morbidity predisposed to by this disease which may be between 25 and 75 million cases.

Malaria has a markedly deleterious influence upon the physical development of both children and adults, in populations residing in localities where they are seriously afflicted with the manifestations of the disease.

The expectation of life in India at some ages is only half that in the United Kingdom, and there appears to be little doubt that malaria, both by its direct and indirect actions, is a very important, if not the most important, factor in causing this deplorable circumstance.

D. THE EFFECTS OF MALARIA UPON SOCIAL, INTELLECTUAL AND POLITICAL PROGRESS OF THE NATION.

'The dwellers in malarious regions, consciously or unconsciously recognising the peril, tend to avoid toil, either of mind or body, if it be so violent that an attack of fever may be expected to follow. In time the impulse becomes stereotyped as a habit, and so, partly for the reason given here, and partly because the energetic migrate to healthier homes, laziness and lack of enterprise are marked characteristics of these unfortunate people. Each generation as it is born is subjected, not only to the same physical surroundings as were its parents, but also to an unhealthy moral atmosphere' (Jones, 1909).

'It is essentially a political disease—one which affects the welfare of whole countries' (Ross, 1911).

'Its effects, moreover, are cumulative. They are handed on from one generation to the next, and become to the community an increasing handicap in all things that make for social development' (Rose, 1919).

'Malaria is a greater foe to civilisation in the tropics than any one other factor' (Bass, 1926).

'All countries are seriously handicapped, and their natural development towards the highest economic, industrial and political efficiency is materially retarded by their malaria' (Hehir, 1927).

'So it would appear that malaria cuts into the progress of man at every stage—preventing, hindering, damaging him at birth, inhibiting his progress physically and mentally by rendering him indifferent to means of defence—producing a vicious cycle, so that many emerge from the early stages of infection lacking energy, initiative, become ruled by habit, hate what is new' (Anderson, 1927).

'Malaria is an indication of conditions of unfavourable social hygiene' (Swellengrebel, 1926).

'The great evil of the fever (malaria) is that it produces degeneracy in races of men who otherwise would be vigorous and progressive' (Nicholls, 1924).

Even in the time of Hippocrates the effects of malaria upon the social, intellectual, physical and political development of the people were recognised. The action of the disease upon the degeneration of important nations is well

illustrated in history. Jones (1909) cites malaria as the chief cause of the change in Greek character which took place in the 4th century B.C. About this time the Greeks became dissatisfied, querulous, and gradually lost their brilliance. Patriotism declined, initiative vanished, vacillation and indecision, fitful activity followed by depression, cruelty and weakness in public life, led to the struggle with Macedonia and the final conquest by Rome. By 300 B.C. the Greeks had lost much of their manliness and intellectual vigour. The decline of the Roman Empire has also been linked up with the spread of malaria, as was possibly the disappearance of the ancient civilisation of Ceylon. Some other workers attribute the decline of Egypt to the same cause.

The deteriorating and obstructive action of this disease upon the social, intellectual and moral advance of any community has been emphasised by many other writers.

Maculloch (1827), the author who first introduced the word 'malaria' into the English language, drew a most graphic picture of the mental and physical degeneracy of the people in the malarious parts of Italy and France in his time. North (1896), in his book on 'Roman Fever', says that malaria in those countries where it prevails to any serious extent 'always will exert a profound influence upon the character of the race, and its position in the human family.....The race, if left to itself, tends towards moral and physical degradation'.

Wise (1920) concludes that one of the principal effects of malaria on the population of British Guiana is its action in producing a diminished mental and moral capacity.

In an Editorial in the *United States Public Health Reports* (1919), it is stated that 'it is well established that wherever malaria prevails, and almost in direct proportion to its prevalence, the population is generally subnormal physically, mentally and economically'. Terry (1929) says, with reference to the African population of the United States of America, 'the malady not only physically incapacitates but dulls ambition, destroys enterprise and lowers morale. The stigma of "laziness" falls upon a malaria-infected population'. Hoffman (1928) points to Jamaica as being 'one hundred years behind in the achievement of its most obvious commercial and social possibilities, largely because of the extensive prevalence of malaria'.

Banerjee (1916) deplores the deterioration caused by malaria in the district of Nadia in Bengal, 'a place of renown in our religious and social history,the birthplace of Chaitanya, the great founder of Vishnuism, the home of Raghunandan, the most illustrious jurist that Bengal has ever produced'.

Apart from its action upon the body, any one who has suffered from malaria, or who has observed closely cases of this disease, cannot fail to have noticed its effects upon the mentality of the sufferer*—mental activity is dulled, irritability of temper is the rule, initiative is lacking, decisions are put off or reached with difficulty, ambition is lost, and depression is a prominent symptom. While such mental states may only be of temporary duration in patients who are able to obtain proper treatment, it is different among the large proportion of the population in malarious areas of India, where few persons ever get such

* For a fuller discussion of the mental effects of malaria, the reader is referred to James (1920) and Anderson (1927).

adequate treatment. One has only to visit a highly malarious village to see these mental symptoms emphasised and multiplied manifold. The end results of this mental state upon the outlook and intellectual development of the people have been vividly described in some of the quotations given above.

While such mental states in the adult may not be of so much importance as a factor in the causation of economic loss with unskilled labour, as is the physical disability, it is otherwise in the case of skilled work. Not only will the quantity of such work be diminished, but its quality will also deteriorate. Rao (1928) points out that 'in a big railway administration with thousands of skilled employees, the mental factor has a very definite significance', and he instances a case where such a state was nearly responsible for a grave accident on the railway line. In speaking of tea estates in Assam, Murphy (1928) also notes that 'loss of efficiency due to malaria is not confined to the manual worker, and it requires emphasizing that impairment of memory, judgment and decision is a frequent result'.

There can be no doubt that in work where large sums of money and great responsibility may depend upon initiative, upon the formulation of a definite and considered policy, and upon rapid and expert decision, the results of such an unbalanced mental state may be productive of serious consequences, financially and otherwise. It must also be remembered that, apart from the sickness of the worker himself, his state of mental anxiety may be determined by serious illness caused by malaria among the members of his family.

The ill-effects of malaria fall most heavily upon the child population. In an Editorial in the *United States Public Health Reports* (1919), it is stated that 'competent investigators are of opinion that the backward condition of school children in the coastal plains of the Carolinas is almost entirely due to the prevalence of malaria'. Leipoldt, Cleaver and Elias (1921) studied carefully the effects of this disease upon the school children of the Transvaal, and considered that children infected with chronic malaria were mentally to be classed as 'feeble-minded'. Leipoldt, in his report on malaria and abnormal children, expresses the opinion that there is a relation of cause and effect between chronic malaria, feeble-mindedness and 'poor whites'.

The disease has a marked effect upon the nervous system and the mentality of the individuals afflicted with it. It is easy to see how a child, whose early years have been marked by a succession of weakening attacks of fever, will probably enter adult life with a debilitated constitution and an ill-educated mind. It cannot be expected that children suffering chronically from this disease, will be mentally capable of obtaining that benefit from literary teaching or other training that healthy children would derive. Apart from the mental state, attacks of the disease will seriously interfere with their attendance at school. It is not difficult to understand how the combined effects of physical weakness and nervous debility must hinder the powers of concentration and retard the normal development of the intelligence of such individuals*.

* 'Public health (in India) has been transferred to self-government. Consequently, the people, who elect their representatives, can provide public health or not as they like. It seems that, in this way, a heavy responsibility has been laid on the shoulders of the people; it has to be faced, and it can be faced in one way only: rendering the task easier of accomplishment by educating the young receptive minds in the schools. *The future of public health (in India) lies in the hands of the younger generation*' (Malaria Commission, League of Nations, 1930).

The mental lassitude caused by the disease tends to become fixed in later life. 'Malaria perpetuates ignorance, and ignorance malaria', while 'malaria begets poverty, and poverty malaria', so a vicious cycle is formed whereby the poor cannot afford to send their children to school for economic reasons, and ignorance and deterioration of the mental state of the malarious population proceeds apace. This unfortunate state becomes more marked as the result of migration. 'Those who leave the country to seek healthier homes are mostly the rich and the intelligent; and so at length there remains but a residue of the poor, the stupid and the unenterprising. Left to themselves, these wretched inhabitants sink into greater degradation, for they are without any to ameliorate their lot by example, precept and active help' (Jones, 1909).

There is no doubt that the education of the people is to be advocated as an important step in reducing the disastrous effects of malaria upon the rural masses of India, who form the bulk of the malarious population. A speech recently made by His Excellency the Governor of the Central Provinces, Sir Hyde Gowan (1934), suggests that the present system of education may not be very helpful in this respect. He says—'It is not my business to criticise the education policy pursued by Government for many years but in this province that policy has had one most regrettable effect, to which no one can be blind. In a land where more than three-quarters of the people are agriculturists, it has drawn the best brains away from the villages into the cities and has taught them to use their pens and their tongues instead of the ploughshare upon which the very life of the country depends'.*

It is obvious that a community, whose mental state is such as that described, is not in a condition to initiate any remedial policy without assistance from outside, nor is it usually in a financial position to do so unaided. 'A seriously malaria-ridden population is incapable, unaided, of maintaining a well-balanced health program. The obvious indication is to get rid of malaria first, put the community on a par with others which fortunately do not suffer from this handicap, and then proceed with normal development in affairs of health and of economic and cultural development' (Cumming, 1927). A similar opinion is expressed by Regnault (1922).

The progress of a nation depends upon the mental and physical vigour of its people. If the younger generation can be protected from the evil effects of malaria, they will be able to take greater advantage of the educational facilities available, and, when they grow up, will not suffer to the same extent from the mental apathy shown by their parents. They will be in a better position, mentally and physically, to initiate and carry out measures for the social, intellectual and political advancement of their community†.

CONCLUSIONS.

Apart from the economic loss caused by the action of malaria upon the mental state of the population, this disease has the deplorable effect of hindering greatly the intellectual, social and national development of any people afflicted by it.

* Jones (1909), in speaking of the decline of Greek civilisation, says 'the presence of vast numbers of slaves not members of households, but gangs of toilers whom the increase of commerce brought into the country, pandered to a foolish pride that looked upon many kinds of honourable labour as being shameful and unbecoming of a free man'.

† *Vide* footnote, p. 425.

E. THE FINANCIAL AND ECONOMIC LOSSES, DIRECT AND INDIRECT, WHICH MALARIA CAUSES TO INDIA.

'It is a self-evident truth that it (malaria) means well for the physician. But for the laboring men it means an immense loss of their time together with the doctor's fee in many instances. If the members of their families other than themselves be affected, it may also mean loss of time together with doctor's fees. For the employer it means the loss of labor at a time perhaps when it would be of greatest value. If it does not mean actual loss of labor to the employer it will mean the loss of efficiency of his labor. To the farmers it may mean the loss of crops by want of cultivation. It will always mean the non-cultivation or imperfect cultivation of thousands of acres of valuable land' (Herrick, quoted by Howard, 1909).

'There is manifested the tragedy of millions of lives lost for lack of knowledge, of naval and military expeditions wrecked, of vast treasures wasted, of notable and promising enterprises abandoned. There is the tragic burden of inefficiency, sorrow, misery and despair, tales of which are scattered through many books, and which has left its mark, not solely in the form of graves, throughout the length and breadth of the tropical world. There is much of tragedy in the shape of lost opportunities, in the strangling hold of vested interests, in the stupidity, folly and pig-headedness, aye, and wickedness, of those who oppose progress, who are deaf to the claims of science, who did their best to set back the clock' (Editorial, *Lancet*, 1924).

'It is evident from this that India has to bear an enormous economic burden from the incidence of preventable disease, which is sufficient to prevent her rising to the position of importance to which her huge population and resources entitle her' (Mackie, 1925).

'In the number of deaths caused either directly or indirectly, the sickness and suffering, the lowered vitality of those afflicted, malaria is without rival. But of even greater moment are the economic losses to the individual suffering with malaria, to industry and agriculture depending on malarial labor, and to the community where malaria prevails' (Fuchs, 1922).

'Malaria, unlike cholera, is not a swiftly killing disease, but no disease is more disastrous to the well-being and efficiency of the population' (Royal Commission on Agriculture in India, 1928).

'The social and economic loss resulting from this enormous amount of (malarial) sickness can hardly be guessed at in view of the inadequacy of our existing statistical information' (Hoffman, 1928).

Apart from any humanitarian aspects of the damage which malaria causes by its mortality, its morbidity and its hindrance to the natural increase of the population, apart from any of its effects in preventing the social and intellectual development of the nation, one must consider the financial and economic losses which this disease causes, both directly and indirectly, to the people of India. Very often it is only by a statement of the last aspect of the subject that the malarialogist and public health worker can hope to obtain any adequate financial aid in his endeavours to ameliorate the ravages of this disease.

Estimations have been made as to the financial loss which malaria causes in different countries. In the United States, it is calculated that each year, in deaths, loss of labour, reduced value of real estate, and in other ways, malaria costs the country \$100,000,000 (Howard, 1909; Wellman, 1913).

In a Medical Bulletin issued by the University of Missouri (1916), it is said that malarial deaths alone cost the United States \$60,000,000 annually. Le Prince (1919) expresses this loss by saying 'the American nation is paying annually 100,000,000 dollars for the privilege of having chills and fever'. Fuchs (1922) states that, in an address before the Senate of the United States in 1916, Senator Ransdell placed the loss from malaria in that country during 1913 at the astounding sum of \$694,907,750. In California alone, the economic loss from this disease is estimated at nearly \$3,000,000 per annum. Fricks (1920) says it causes a greater economic loss to the malarious sections of the South than all other preventable diseases combined—approximately a billion dollars a year. The loss to the State of Louisiana alone is given at \$1,000,000, and this is considered a low estimate (Wright, 1922). Williams (1935) calculates that in the State of Georgia at least \$1,500,000 is spent in medical aid, and the 'lowered output represents a loss to the state of not less than \$15,000,000' per annum,

Ross (1911) stated that Bolton calculated malaria cost the island of Mauritius, with its population of 383,000, about Rs. 10 lakhs per annum. A correspondent in the *Journal of the American Medical Association* (1930) writes to say that the economic toll on the populations of Natal and the Transvaal is abnormally heavy. 'In the absence of proper data it is impossible to calculate this loss accurately, and the estimate of £6,000,000 a year, as the loss sustained by interference with industrial labour on account of malaria, must be regarded as a mere estimate, probably below the actual figure'.

These statements show the very heavy financial losses which malaria causes in other countries, and the serious notice which is being taken of them. It must be remembered that the population and the amount of malaria in India are very much greater than in any of the countries mentioned and so the economic loss must be proportionately greater.

Several estimates have been made as to what malaria costs the Indian Empire financially. Ross (1911) says 'Colonel W. G. King, the distinguished Sanitary Commissioner of Madras and Burma, informs me that, from estimates which he has made regarding the cost of death and sickness in India, he finds that the death of an adult costs Rs. 20; of a child Rs. 7; while a month's sickness of an adult costs Rs. 9 and of a child Rs. 4-5. As there are quite 1,000,000 deaths from malaria each year in India, with a corresponding amount of sickness, the total cost of the disease may be roughly computed from these figures'.

Colonel King, in an unpublished memorandum in 1911, states that, taking the figure of infected persons at 100 million per annum and assuming that adult males of the lowest labouring class earn wages at the rate of Rs. 5 per mensem between the ages of 15 and 55; that 'no vested value in the life of the producer is allowed; that—as is not the case—females be held not to be wage-earners, it will be found that by "unproductive" expenditure India loses Rs. 25,55,75,810 per annum' (i.e., about £19 million sterling). 'This is admittedly a rough calculation; yet it probably underestimates the facts, seeing that the malarial subject may have repeated attacks of fever annually, and is doomed by the direct and indirect results upon his system to a condition of reduced resistance to other diseases'. 'Experience shows that it would be legitimate to take cognisance of morbidity and consequent disability from labour by other causes to the extent of 20 per cent (percentage constitution of morbidity population)—thus raising the total to Rs. 30,44,80,735 per annum' (i.e., about £23 million sterling). 'The latter aspect of the matter is of special moment in reference to the swelling of pension charges, on account of military and civil officials, who have, in age and service, broken down prematurely'*

Balfour and Scott (1924) remark that 'it is calculated that the direct annual cost of sickness and death resulting from malaria in India is between fifty and sixty million pounds sterling, and this, be it noted, is but the *direct* cost, taking no account of the even greater indirect losses due to the resultant impaired productive power of labour'. Rose (1919) makes a similar statement. Chopra (1933) and Choksy (1933) place the annual direct loss to this country

* Since Colonel King wrote this note the cost of living and of wages has increased very greatly. The *Indian Year Book* for 1929 says that, in the Bombay Presidency, the wage index of agricultural labourers has doubled between 1913 and 1917, so at the present time any calculation on the basis given by Colonel King would be at least half as much again.

from malaria at £25 million sterling, and the former states that the 'economic loss and consequent penalty which has to be paid by the country as a whole is tremendous'.

It is said by Bentley (1925) that, in Bengal alone, millions of lives have been sacrificed, thousands of crores of rupees have been lost. The people are sunk in poverty, and the vast population of them suffer each year from recurring attacks of malaria.

In our present incomplete state of knowledge, it is impossible to give any accurate figures as to the magnitude of the financial and economic losses which malaria causes to India. There are, however, certain data available, from which it appears possible to obtain some indication of the amount and immensity of the loss suffered in certain ways.

In discussing the problem, it seems most convenient to divide the question into the following headings :—

- (I) General considerations.
- (II) Losses to the individual and the family.
- (III) Losses to the community.
- (IV) Losses to agriculture, industry and commerce.
- (V) Losses to public administration and to Government.

(I) GENERAL CONSIDERATIONS.

The influence which malaria has had in shaping the history of the world is enormous. This is the disease which arrested Mark Antony, which was probably responsible for the deaths of Alexander the Great and of Cromwell, which hindered the Crusaders, and contributed largely to Napoleon's failure in Egypt and Palestine. To it has been also ascribed a marked action in determining the fall of many of the ancient civilisations, such as those of Etruria, Greece and Rome, Egypt and Ceylon.

The main factors influencing the financial and economic losses which malaria causes to India, may be discussed under the following headings :—

- (a) Financial and economic losses directly dependent upon sickness.
- (b) Financial and economic losses due to conditions of inefficient, deficient and expensive labour.
- (c) Financial and economic losses due to retardation of, and interference with, agricultural, commercial and industrial progress.

Although the effects of malaria may be discussed under these headings, it is evident that no clear-cut divisions can be made between them and that the different causative factors are usually intimately mixed.

(a) FINANCIAL AND ECONOMIC LOSSES DIRECTLY DEPENDENT UPON SICKNESS.

These losses include the cost of illness—hospitals, doctors' fees, nursing, medicines, etc., the cost of sick leave, the loss of wages while sick, the diminished labour output and impaired efficiency resulting from sickness, the expenses due to deaths and funerals, etc., etc.

These losses fall very heavily upon the individual and the family, and are discussed in detail under that heading in a later part of this article [*vide* Section E(II)]. Many of these factors have also important influences upon the

financial prosperity of the community, of industries, commerce and agriculture, and of Government.

(b) FINANCIAL AND ECONOMIC LOSSES DUE TO CONDITIONS OF
INEFFICIENT, DEFICIENT AND EXPENSIVE LABOUR.

'The effect of the disease (malaria) on the people is to unfit them for labour, to cause loss of time, loss of money and generally to diminish their producing power' 'Thus, other things being equal, a malarious country cannot hope for the same rate of progress as one which has not to pay this heavy tax upon its energies' (North, 1896, quoted by Anderson, 1927).

'There are few diseases which affect the working strength of labour gangs so much as malaria' (Daniels and Wilkinson, 1909).

'It is not in the death rate itself that malaria becomes of importance. It is in the days of unproductive labor and labor shortage when one's burden must be carried by others that malaria puts a drag on the community' (Carter, quoted by Johnson, 1926).

'Serious as they are, the losses suffered by the individual sick with malaria are less significant than those sustained by industry and agriculture in the South. To such enterprises malaria spells inefficient labor, a costly labor turnover, a scarcity of labor often when most needed, with a consequent curtailment of production or of crops and an expensive idleness of machinery or farm hands' 'Probably the heaviest tax imposed by malaria is from inefficient labor. Hands that receive full pay while working with only a feeble effort, place a burden upon the manufacturer, railroad and plantation owner alike' (Fuchs, 1922).

The prevalence of malaria in a community has a very damaging effect upon its economic progress, even when other natural conditions are highly favourable. In localities where the incidence of the disease is relatively low, the effects may be small, but where there is a high incidence the results may be disastrous to the prosperity of the community. During the Punjab epidemic of 1908, Christophers (1910) records that in Amritsar, for example, for many weeks the ordinary business of the city was interrupted, labour could not be obtained, transport was disorganised, and even food vendors ceased to carry out their trade. A similar state of affairs occurred during the epidemic in South Russia in 1922-23 and the effects of the recent Ceylon epidemic have not yet faded from memory. Marchoux (1925) says that 'Corsica and all our (French) colonies are subject annually to such extreme epidemics that their economic development is paralysed'.

Such epidemics cause a disastrous and paralysing effect on trade and industry for the time, and give rise to a disorganisation from which it takes a long time for the community to recover. This general embarrassment is usually but a temporary set-back, and the effects of such epidemic outbreaks are small as compared with the cumulative losses which continuous endemic malaria causes to the community.

To the independent worker, malarial sickness means that he is unable to work for some days during each attack of the disease, and the infection also results in a diminished capacity, both mental and physical, for performing his duties when he is able to return to them. His efficiency is lowered, his output is smaller, and its quality poorer, than that of a healthy person. Such factors also affect the employer of labour very seriously, for he does not get full value for the wages which he pays. The depletion of his staff from sickness may result in the disorganisation of important branches of his industry*, may result

* This may be the case especially in localities where agricultural operations are governed largely by seasonal climatic conditions of which advantage must be taken before the opportunity is lost.

in machines lying idle, crops uncut or untended, etc., and so a loss of interest earned upon the capital invested.

A large turnover of staff often leads to a scarcity of labour and gives rise to difficulty and expense in recruiting sufficient workers to meet requirements. It frequently makes it necessary to pay a higher rate of wages than the market ones, in order to induce workers to accept employment in unhealthy localities. The labour turnover may be so great that in some industries it has been found necessary, especially in the case of skilled and semi-skilled workers, to carry on the pay rolls a large excess of personnel, or even in some important undertakings to reduplicate the staff, in order that at least one out of every pair may be available for duty (*vide* 'Railways').

All these factors mean an increased cost of production, often a diminution in both the quantity and quality of the output, and thus an inability to compete in the open market with the products of a more healthy place. This leads to a loss of market, diminished prosperity, and so increased financial stringency, which may eventually lead to an abandonment of the undertaking.

In countries where malaria is widespread and severe, this interference with labour must give rise to serious financial loss not only to the independent worker but also to the employer of labour. These points are discussed in greater detail in dealing with different industries.

(c) FINANCIAL AND ECONOMIC LOSSES DUE TO THE RETARDATION OF,
AND INTERFERENCE WITH AGRICULTURAL, INDUSTRIAL AND
COMMERCIAL PROGRESS.

'It (malaria) means a listless activity in the world's work that counts mightily against the wealth-producing power of the people' (Herrick, quoted by Howard, 1909).

'The west coast of Africa, portions of India and many other tropical regions have always, at least down to the present period, been practically uninhabitable to civilised man, owing to the presence of pernicious malaria. The industrial and agricultural development of Italy has been hindered to an incalculable degree by the prevalence of malaria in the southern half of the Italian peninsula as well as in the valley of the Po and elsewhere' (Howard, 1909).

'Malaria is the great enemy of the explorer, the missionary, the planter, the merchant, the soldier, the farmer, the administrator, the villager and the poor, and has, I believe, modified the world's history by tending to render the whole of the tropics comparatively unsuitable for the full development of civilisation' (Ross, 1911).

'All malarious countries are seriously handicapped and their natural development towards the highest economic, industrial and political efficiency is materially retarded by malaria' (Hehir, 1927).

'It is not going too far to say that *malaria eradication is essentially a labor problem of the first importance*; that an enormous amount of labor insufficiency due to malaria continues to hinder the progress of semi-tropical and tropical countries, which, if brought under control and completely done away with, must needs assist profoundly in the reclamation of the tropical regions for the practical needs of the world at large' (Hoffman, 1928).

'Malaria is beyond dispute the greatest natural obstacle to the economical and humane development of the Malay States and equally concerns all classes of the community' (Hoffman, 1928).

'The other republics of America as well as ours have serious malaria problems which interfere with their normal development, with industrial development and agriculture' (Le Prince, 1932).

'There is no doubt, however, that malaria exercises a disastrous influence on the economic life of nations and individuals' (Celli, 1933).

It is beyond dispute that malaria is one of the greatest, and probably the greatest, obstacle to the economic development of the natural resources of large tracts of India. The fertile lands of the Terai of the United Provinces cannot be exploited on account of this scourge. The mineral wealth of Orissa and other areas cannot be developed to its full extent because of the effects of malaria. Many agricultural industries (tea, coffee, rubber, etc.) are very seriously hampered by the action of this disease. These are but a few examples of the loss which malaria causes by preventing the proper development of the resources of the country.

From its effects on the labour problem, which have been mentioned above, it raises the cost of production of foodstuffs, materials, goods and manufactured articles. It often interferes with the transportation of these products to suitable markets, and so raises the cost of such carriage.

These difficulties may make it impossible for industries in such areas to compete in the open market with the products of more favourably situated places. The economic losses attendant upon the presence of a marked prevalence of malaria may, therefore, give rise to an absence or retardation of the development of natural resources, which, but for this disease, would form great national assets, and would add very much to the wealth, prosperity and happiness of the country as a whole.

As noted in the quotations at the head of this section, this obstructive action of malaria has been felt in many other countries of the world where this disease prevails. Celli (1933) speaks of the prosperous agricultural and industrial life in the northern parts of Italy, as compared with the inferiority of such enterprises in the southern areas. He points out that malaria is mild in the former areas, while it is severe in the latter, and attributes these economic differences to the action of this disease. Hoffman (1928) believes that the commercial and social possibilities of Jamaica are very seriously hampered by the presence of malaria in that island.

India was unusually prosperous during the War. This was due to a large extent to an increased demand for her products. This abnormal state of affairs arose from an increase in the cost of production in other countries, and because very large numbers of their population were engaged in the unprofitable pursuit of war, thus being unable to supply the products in the same amounts, and at the same rates, as under normal conditions. Under such circumstances, India was able to become a more serious competitor in the world's markets, in spite of the handicap of her malarial conditions. With a return to more normal conditions and the removal of the burden of war from other countries, India's load of malaria again places her in an unfavourable position, and her temporary prosperity has largely faded away, giving rise to conditions of financial stringency.

Apart from its effects in retarding development, malaria has been responsible for the abandonment of many promising enterprises.

'Very malarious places cannot be prosperous; the wealthy shun them; those who remain are too sickly for hard work; and such localities often end by being deserted by all save a few miserable inhabitants' (Ross, 1911).

'The group of maladies called malarial are perhaps the most widely distributed and the most disastrous in their effects of all diseases to which man is liable. Their presence renders large portions of the earth's surface absolutely uninhabitable' (North, 1896, quoted by Anderson, 1927).

'Malaria in fact rendered vain, time and again, every attempt made to colonise the Roman Campagna and other districts' (Celli, 1933).

One never sees homes completely abandoned on account of enteric fever or tuberculosis, or even due to cholera and plague for more than a short period, but hyperendemic malaria has and does cause this desertion. It is probably true that malaria is the only disease of man, possibly excepting African sleeping sickness, which can reach such proportions as to render an area actually uninhabitable.

As mentioned previously, considerable evidence has been accumulated which suggests, very strongly, that the decline of many of the ancient civilisations of the world was due to the detrimental effects of malaria, or, at least, that this disease was a marked factor in producing their decadence.

Nicholls (1921) states that 'the ancient cities of Ceylon owed their rise to the field labourers who built the tanks and cultivated the rice fields, for at this time the country was self-supporting; and it is probable that the decline commenced among the lower classes, and it is reasonable to suppose that the cause was a devitalising disease which impaired their energies' 'The gradual fall of these people was due to the importation from India of malaria and possibly also of the anopheline mosquitoes, the conveyors of the disease. When once malaria was established the people and their culture would drift to the less malarious parts, and that is what happened' *.

Bentley (1925) (pp. 1—3; pp. 24—26) gives numerous instances where malaria and agricultural decline have been associated in other parts of the world. Anderson (1927) mentions especially the I-land of Brioni in the Adriatic Sea, once an important province of the Roman Empire and of Venice, and still showing vestiges of Roman civilisation, and which became uninhabitable for centuries because of malaria. Howard (1909) speaks of large tracts of highly productive agricultural land in the Southern States of America abandoned on account of the severity of malaria.

The malarious parts of India are not without many striking examples of localities where malaria has given rise to the depopulation and practical abandonment of previously prosperous areas. Attention has been drawn to these disasters more especially in Bengal. Banerjee (1916) paints a moving picture of the deserted homesteads and the ruined buildings of once healthy districts in that province. Bentley (1925) also draws attention to this devastation, while Brahmachari (1923) mentions that the depopulation of areas, where epidemic malaria occurs, is aggravated by the exodus of panic-stricken people.

Clemesha (1917) speaks of Ennur near Madras City as being previously a health resort and then abandoned because of malaria. This place also attracted the attention of the Malaria Commission of the League of Nations (1930) during their tour in this country. The Commission says :—

'The coastal villages in and behind the low sand dunes north of Madras present an interesting malaria problem' 'since a deliberate attempt was made to prevent malaria. This attempt, however, was discontinued when the area came under self-government' 'Stephens and Christophers long ago proved *A. culicifacies* to be the sole carrier there. A splendid opportunity is thus afforded to carry out species sanitation and at the same time right an obvious wrong, for the people suffering most from malaria are the fishermen whose villages on the dunes are nearest to the plantations. The farmers and the people tending

* See pp. 456-460.

the trees live further away and suffer less, and the owners live in Madras and suffer not at all. The opportunity has been recognised and then neglected, and things are left as they were before'.

Marjoribanks (1914), in his survey of Salsette Island, near Bombay, notes 'the presence of lines of villages, deserted about a generation or two ago, along the slopes of the hills, clearly shows that at one time the countryside could not have been nearly so malarious as it is now' 'One very depressing feature of the evidence is that it shows that in Salsette Island malaria is much worse than it used to be a generation or two ago, and this without any change in the physiography of the district'.

In the Central Provinces, Kenrick (1914) also stresses the abandonment of villages in fertile areas on account of malaria.

'In some cases malarious villages with good land were taken up by two or three families in succession, only a few months sufficing for malaria to clear away each whole family' 'Piplod was once a flourishing village of some 180 houses; at present only 68 houses are occupied, and much of the cultivated areas has been replaced by scrub jungle. There is a universal complaint of fever and people are said to be leaving the village yearly; the children have a spleen rate of 60 per cent, and many of the adults are affected' 'The hilly country of the Melghat, therefore, is a centre of hyperendemic malaria, and, as long as the present strict reserves are maintained, will not only be closed to all immigration, but gradually become more and more depopulated, as the aborigines emigrate to the more congenial surroundings of the cleared areas, on the border of the reserve' 'The plateau itself is now almost uninhabited, only two or three villages remaining, but the sites of old and forsaken villages in many directions show that at one time there was considerable cultivation'.

These are but some examples of the disastrous effects of malarial prevalence upon the development of the natural resources of the country. Others in relation to special industries—agriculture, railways, mining, etc.—will be mentioned in later sections of this paper.

(d) SUMMARY.

A general discussion has been given of the financial and economic losses which malaria causes through sickness, through the production of conditions of inefficient, deficient and expensive labour, and through its action in retarding and preventing the fullest development of the natural resources of those countries or localities where the disease is prevalent.

It is necessary, however, to consider in greater detail the financial and economic losses which the disease causes to the individual, the family and the community, as well as to the agriculture, industry and commerce of this country. These are dealt with in the succeeding sections.

(II) FINANCIAL LOSSES TO THE INDIVIDUAL AND TO THE FAMILY.

'It is a case of tired minds in tired bodies, and naturally under such conditions there can be no efficient labour. Looking at things from a mundane point of view, there is a loss of time and money. The worst of it is that this goes on year in and year out, so that the burden of expense due to lack of efficiency, hospital charges, doctor's fees, drugs, and other causes, attains surprising dimensions' (Balfour and Scott, 1924).

'The common people and the laboring class in general are direct losers in the way of time lost, doctors' bills, medicine, and lowered vitality. They also suffer an indirect loss due to the poorer economic condition of the community. The direct loss is by far the smallest loss caused by malaria' (Johnson, 1926).

Apart from the annoyance, suffering, misery and death caused by attacks of malaria, the financial loss to the individual and the family must be very great.

Ross (1911) says that Colonel W. G. King, I.M.S., estimated that, in India, a month of sickness in an adult cost Rs. 9, and in a child Rs. 4½. If we agree that only 33 per cent of the sickness falls upon adults and the rest on children, that the duration of the sickness is 14 days yearly*, and that 100 million cases of malaria occur annually, the direct loss to the individual, or, to the family, can be calculated at about Rs. 3,000 lakhs, or £23 million sterling per annum.

The financial loss may be considered under the following heads :—

- (a) The cost of medical attendance, etc.
- (b) The loss of time, efficiency, wages and employment.
- (c) Losses due to deaths, and
- (d) Miscellaneous.

(a) COST OF MEDICAL ATTENDANCE, ETC.

No reliable figures are available of the sums which are spent annually by private individuals on medical attendance, on drugs, on nursing and the lost wages of family members who act as sick attendants, on extra food, etc.

In the United States of America, Fuchs (1922) estimates that the labourers on one railway in a malarious area spend up to \$30 per annum, with an average of \$5 to \$10 for quinine and 'chill tonics'. Bass (1918) estimates that in one malarious county in the same country an average of \$2½ per annum is spent in this way, while in another area an average of \$17.30 was the combined expenditure for medical service and drugs per case.

Colonel W. G. King, I.M.S., in an unpublished memorandum prepared in 1911, estimates that, during an illness lasting 14 days, the cost of extra necessaries and food, medical attendance, medicine, etc., in the case of an adult of the lowest labouring class in India, was Rs. 2 and of a child Rs. 1½. If one takes the figure of 100 million cases of malaria in British India annually, and a cost of only Rs. 1½ per case, this means that the losses of the family and the individual amount to Rs. 1,500 lakhs per annum, or about £12 million sterling for medical attendance, etc., only. The figures used by Colonel King were estimated nearly a quarter of a century ago, and since then these must have risen very considerably, so such an estimation must be low rather than excessive.

It must also be remembered that such figures do not include any losses directly or indirectly due to the need to nurse a sick person, more especially if this be a child.

Bentley (1908) states that on a tea garden in the Bengal Duars with 1,350 working coolies, he frequently saw during the rains 50 to 70 women attending hospital on one day, because of fever in their infants or children. Rice (1931) found in the same area that many women, who worked regularly during the cold weather, rarely turned up for work during the plucking season. This he considered to be readily explained by the fact that young children, before they become immunised, suffer intensely from malaria, thereby requiring nursing, the result being that mothers absent themselves from work for this purpose. Macdonald and Chowdhury (1931) say 'it is the rule for the mother

* *Vide* pp. 437-438.

to remain away from work if her child is ill, and in the case of any other person being seriously ill, one other person absents himself to care for the invalid'. A similar statement is made by Forsyth (1928).

Van Dine (1916) studied the malarial conditions among an agricultural population of 299 persons in the Southern States of America. He found that 166 cases of malaria occurred among 138 persons, and the total loss was 1,066 days, of which 385 were lost by non-malarious members of the community in nursing sufferers from the disease. Fuchs (1922) found that attendance upon a malarial patient frequently involves an absence from productive work on the part of other members of the family. While the loss is undoubtedly considerable, it is difficult to estimate. He reports that in one plantation in the United States the loss of time to wage-earning members of the family due to nursing duties was about 3 days per attack.

Taking 3 days as the average loss of time to a wage-earning adult for nursing an attack of malaria in another member of the family, and that even an average wage of Rs. 5 or its equivalent is earned per mensem (*vide infra*), the financial loss from this cause would be another Rs. 500 lakhs or £4 million sterling. This calculation is based on the assumption that *only* one attack of malaria occurs in each individual annually, which is not in keeping with the known relapsing properties of this disease, especially in untreated cases.

These figures appear to indicate that, on the lowest possible estimates, the cost to the individual and the family for medical attendance, etc., is at least Rs. 2,000 lakhs or about £15 million sterling per annum.

(b) LOSS OF TIME, WAGES, EFFICIENCY AND EMPLOYMENT.

'By far the heaviest tax on the individual who has malaria is from loss of time during illness. If the patient is of working age, the loss of wages may be of vital importance to himself and his dependents. If of school age, the loss to the child is not measurable in dollars and cents, but may count for much in a rural schooling that is none too adequate at best' (Fuchs, 1922).

'Naturally, the inhabitants of malarious places tend to avoid fatigue, and to become sluggish and unenterprising. A habit of laziness is gradually formed' (Jones, 1909).

The question of the loss which India incurs from preventable disease was discussed at the 2nd All-India Conference of Medical Research Workers in 1924, and the following resolution was passed :—

'That this Conference believes that the average number of deaths resulting every year from preventable disease is about five to six millions, that the average number of days of labour lost by each person in India from preventable disease is not less than a fortnight or three weeks in each year, that the percentage loss of efficiency of the average worker in India from preventable malnutrition and disease is not less than twenty per cent. . . . The Conference believes that these estimates are under-statements rather than exaggerations'

There is no doubt that of all the diseases from which the inhabitants of India suffer, malaria, on account of its widespread prevalence and its chronic relapsing nature, is responsible for the greatest amount of sickness and lessened labour efficiency.

In the evidence given before the Royal Commission on Agriculture in India (1928), it is stated that in the United Provinces one-fourth of the total population of 45 millions get two attacks of malaria each year, and only one per cent receive proper quinine treatment. Twenty-five per cent of the

population are totally incapacitated for two months, besides having a lower vitality for the rest of the year.

In Bengal, Bentley (1925) remarks that 'there are relatively few areas at present in which the majority of the population does not suffer from an attack of malaria at least once a year. But in the unhealthy districts a vast proportion of the population is composed of chronic sufferers from the disease, who are a prey to recurring attacks of fever for many months every season'.

Christophers (1911*a*) in reporting upon the epidemic of 1908 in the Punjab, states that 'in parts most severely affected the epidemic is stated to have prostrated the entire population. In Amritsar, for example, for many weeks the ordinary business of the city was interrupted'.

It is extremely difficult to form any precise estimate of the financial loss to India from the sickness and inefficiency caused by this disease. Even when one makes a calculation from the days lost due to sickness, this does not include the probably greater financial loss due to the inefficient labour performed by the worker during the period of convalescence or latent illness, nor the loss due to sickness and inefficiency caused by other diseases to which the malarial infection predisposed the sufferer.

(1) THE NUMBER AND VALUE OF DAYS OF WORK LOST DIRECTLY DUE TO MALARIAL SICKNESS.

In making any calculation of the value of the days lost due to malaria, it is necessary to have some idea of the average number of days lost yearly by each sufferer from the disease, and the average value of these days.

Workers in different countries have given figures showing the number of days actually lost due to attacks of malaria.

Senior-White and Newman (1932) give figures of the losses on the Adriatic Railway in Italy during the 10 years from 1881 to 1890. These figures show an average loss per annum due to malaria of 15 days per worker or 19 days per case. In Louisiana, Hoffman (1928) says that a study of 12 agricultural families in the Southern States of America showed a loss of 7 weeks per family during the crop-growing season. Fuchs (1922) states that in the same country adults lost 15½ days. Old (1922) gives 7 days as the average lost on the Central of Georgia Railroad per case of malaria, while an Editorial in the *New Orleans Medical and Surgical Journal* (1923) states that an industrial institution in Louisiana reports an average loss of 8 days. Thézé (1916) reports that in the French Guiana Penal Settlement the average annual loss was 25 days per individual. In Palestine, the minimum time lost was found by Kligler (1924) to be 3 days per treated patient, while Ross (1911) states that Bolton found in the sugar plantations of Mauritius 15 per cent of the adult males were incapacitated for 3 months in the year. This is an average of about 13 days per person.

Several estimates and observations have been made in different parts of India as to the loss of work caused by malaria.

Bentley (1908) says that, during the manufacturing season in the tea estates of the Bengal Dooars, many of the coolies were off work from 1 to 5 days. In the same area, Rice (1931) gives figures which indicate that, apart from any loss of efficiency, about 4 per cent of working days are lost due to malaria. This would equal about 14 days in the year. Macdonald and Chowdhury (1931) report that on certain tea estates in Assam the loss was about 2 days per person per annum. Manson (1931) studied the population in another group of tea gardens, and records an average loss of 3½ days per person annually, the limits in different gardens being 15.77 and 0.63 days. Fraser

(1934) reports that as a result of anti-malarial measures, the average annual sick days per person on certain tea gardens in the Assam Valley fell from $14\frac{1}{2}$ to $8\frac{1}{4}$. Even if we assume that all sickness from malaria had been eliminated by the measures taken, the previous loss due to this disease must have been at least 6 days annually per individual. In the same province, Gupta, Das and Majumdar (1932) consider that each attack of malaria entails a loss of one week of work. Bentley (1911b) says the average loss of duty among policemen in Bombay was about 7 days for an attack of malaria, while Senior-White and Newman (1932) give the average loss among railway employees as 8 days. Clyde (1931) found that, among a non-immune population introduced into the highly malarious Terai of the United Provinces the average monthly percentage loss of man hours was 10.8 per cent.

This subject was very carefully studied by Van Dine (1916), who found that the actual time lost due to an attack of malaria was about 6.42 adult days among the agricultural labourers in the Southern States of America. In the Indian Army the time lost by a soldier is almost the same.

In the evidence given before the Royal Commission on Agriculture in India (1928), it was stated that 25 per cent of the population of the United Provinces are *totally* incapacitated for 2 months every year by malaria. This would correspond on an average to about half a month per head per annum of the *whole* population of these provinces, which are probably not on the whole very much more malarious than some of the other Indian Provinces.

Practically all the above results are based upon the study of patients who receive specific treatment of some sort. On the other hand, it is probable that about 90 per cent of the malarious sick of India receive no such treatment to cut short their attacks. Even on these figures, if each sufferer in India has a minimum of two attacks each year, the direct loss would not be overestimated if taken at 14 days per annum. This was the figure used by Colonel King in his unpublished note of 1911.

From the data available it would, therefore, appear to be a conservative estimate to assume that the number of adult days lost per head of the *infected* population each year is about equal to half a month at least.

Before one can make any estimate of the cost to the family and the individual of days of work lost, due directly to malarial sickness, it is necessary to consider what is the average annual income per head of population in this country. Lord Curzon in his Budget speech of 1901 gave the average income in India as Rs. 30 per head per annum. Since that time there is no doubt but that the average income has increased. Horne (1918) estimated the average annual income in India as Rs. 42 per head, while Slater (1918) estimated that in the Madras Presidency it was on an average over Rs. 70. The *Hindustan Times* in its number of 23rd September, 1934, gives the annual Indian income as Rs. 45, and quotes the following estimates made in recent years by different authorities :—The Hon'ble Sir B. N. Sharma in 1911 at Rs. 86; Professor K. T. Shah in 1921-22 at Rs. 46; the Simon Commission in 1928 at Rs. 110; and Sir M. Viswesariah in 1933 at Rs. 60. Joshi (1933) in the *National Call* of 22nd June, 1933, is reported to place the average income at less than £4 sterling (Rs. 53). In order that a minimum estimate may be used in our calculations, let us take the lowest figure given as the basis of our estimates, namely Rs. 45 per head per annum.

It is difficult to find exact figures of the value of the average wage or its equivalent earned by the adult worker in India.

Bentley (1911b) gives the average earning power of workers in Bombay City at Rs. 20 per mensem. At a later date, Covell (1928) considered that Rs. 30 was a very low estimate. Rice (1931) states that, on the tea estates of the Bengal Duars, the average pay *plus* perquisites of a working coolie are equivalent to about Rs. 26 per mensem. Gupta, Das and Majumdar (1932) think that Rs. 30 is a moderate estimate of the average earning capacity of each individual in a forest area in Assam. Lal and Shah (1933) give the average income per family in a water-logged, poor and very malarious, agricultural area in the Punjab as between Rs. 25 and Rs. 30 per mensem.

From the figures given by various workers, it does not seem to be an over-estimation to consider the average earning capacity of an adult male to be equivalent to a sum of Rs. 10 per mensem, and of an adult female to be Rs. 5. The adult working population of British India (*i.e.*, those between the ages of 15 and 50 years) was found at the last census to consist of about 70 million males and 66 million females. As malarial illness appears to fall equally on both sexes,* and as adult males are in excess of females, the use of an average earning capacity of Rs. 7½ per mensem for all adults, irrespective of sex, would seem to be a modest sum upon which to base our calculations. Such a figure would also coincide with the lowest estimate of an average income per head of Rs. 45 per annum, when one assumes that only persons between the ages of 15 and 50 years are wage-earners†.

If one take the average time lost each year, as the direct effect of malaria, to be half a month, an estimate of the financial loss to the individual and the family can be made from the data given.

(i) In the earlier part of this paper, it has been shown that probably at least 100 million persons suffer from malaria each year in India. It has also been pointed out that the fatal effects of this disease are more marked among the children than among the adults. The latter does not necessarily mean that a large number of children are infected, but that those infected are more liable to succumb to the effects of the disease than are adults. However, again to make our estimate conservative, one may safely assume that at least one-third of the infected population are adults. This means that 33 million adults suffer from malaria each year, and lose an average of half a month of working days from this cause. If the value of a month's work is an average of Rs. 7½, this means a total loss of about Rs. 1,240 lakhs per annum, or nearly £10 million sterling.

(ii) The estimate may be made in another way. The value of the average income per head of population in British India is at least Rs. 3¼ per mensem. If each of the 100 million sufferers loses half a month's income the loss is about Rs. 1,900 lakhs. If one accept the higher annual income of Rs. 110 given by

* Malaria falls mainly upon the rural population of India, and as 80 per cent of the total population of this country are engaged in agricultural pursuits, these are the individuals most heavily afflicted. Under such conditions both the men and the women are engaged in field work, so both these sexes must be taken into account in estimating the loss of work.

† Half the population of British India are adults between the ages of 15 and 50 years. For the purpose of our estimates, these only have been taken as wage-earners. In order that our estimates may be on the conservative side, persons above and below these ages have been excluded, although very large numbers of them earn at least half the estimated wage noted above.

the Simon Commission the loss would come to about Rs. 4,600 lakhs, or about £35 million sterling.

(iii) The loss may also be calculated by a third method. According to Carter (1919) it has been estimated that a death from pneumonia corresponds to 125 sick days distributed among the population, i.e., working days lost to the community. A death from enteric fever is equivalent to 400 to 500 days, while one from malaria means a loss of 2,000 to 4,000 days. This loss may really be doubled or trebled, for a man infected with malaria is frequently unfit to perform his full work for half his time. This aspect of the problem will, however, be discussed later.

From the figures given earlier in this paper (pp. 245—251), it is seen that probably about 30 per cent of the deaths from malaria in India occur between the working ages of 15 and 50. This means that each year about 300,000 individuals die between these ages in British India, as the direct result of malaria. If each of these deaths is equivalent to even 2,000 working days distributed among the population, the loss equals about 20 million working months per annum. Taking the average value of an adult's work to be Rs. 7½ per mensem, the sum lost is about Rs. 1,500 lakhs each year, while if each death be taken as equivalent to 4,000 sick days, the loss would be about Rs. 3,000 lakhs, or £23 million sterling.

(iv) If we take the total of 1 million deaths, and calculate each as being equivalent to an average of 2,000 working days lost to the community, with an average annual income of Rs. 45 per annum the loss would be about Rs. 2,500 lakhs. If the working days lost be taken as 4,000, then the latter sum is doubled.

Summary.

From the figures available it is certain that the very heavy financial loss is incurred by the individual and the family due to actual days of work lost from malarial sickness. This loss to the inhabitants of British India may be as low as Rs. 1,240 lakhs per annum. Such an estimate is a very conservative one, and the true figure is probably two, three or four times this sum. It is to be noted that these estimates do not take into account any lost efficiency of the worker during the days preceding or succeeding his attacks of the disease, nor time lost by persons who are absent from work to act as sick attendants to the sufferer, nor time lost due to sickness from other diseases, contracted as a result of antecedent malaria.

(2) LOSS OF EFFICIENCY CAUSED BY MALARIAL SICKNESS.

'The effect of malaria in lessening or destroying the productive capacity of the individual is obviously of the utmost importance, and upon the population of a malarious region is enormous, even under modern conditions' (Howard, 1909).

'It is not in its death rate that the gravest injury of malaria lies; it is in its sickness rate, in the loss of efficiency it causes rather than in the loss of life' (Carter, 1919).

'If malaria be present you lose efficiency, not only from malaria but from other diseases' (Watson, 1933).

It must be remembered that malaria is essentially a chronic relapsing disease, especially under conditions where efficient treatment is not available. Continued infection with malaria often means that, even in the absence of acute

attacks of the disease, the sufferer is not in good health, and his capacity for work, either mental or physical, is much less than that of a healthy person. This disability is especially liable to become manifest under conditions of stress. As was discussed in connection with economic stress, the loss of wage-earning or productive capacity very often leads to a diminished power to buy food, and so to malnutrition, or even semi-starvation. This sets up a vicious cycle leading to still greater loss of efficiency.

As was pointed out by Fuchs (1922), 'probably the heaviest tax imposed by malaria is from inefficient labour'. As soon as his acute attack is over, financial stringency urges the patient back to work in most cases, but the output of the work performed by him is very much less than that of a healthy man.

It is very difficult to make any estimate of the financial loss due to the disability and lowered efficiency of work performed by a malaria-stricken population.

In certain towns in America, where vigorous anti-malarial campaigns have been carried out, employers of labour report an increase in efficiency among their workers varying from 10 to 40 per cent, as the result of these measures.

In the United States, several workers have made calculations as to the amount of labour inefficiency caused by malaria. Howard (1909) states that a man may suffer from malaria throughout the greater part of his life, and his productive capacity may be reduced from 50 to 75 per cent, and yet ultimately he may die from some entirely different disease. 'It will not be an exaggeration to estimate that one-fourth of the productive capacity of an individual suffering with an average case of malaria is lost'. He also quotes the experience of Dr. Otis Smith, who found that the efficiency of survey parties in malarious places was reduced 'at a conservative estimate, by 25 per cent'.

Hermes (1913) says that, in malarious parts of the State of California, the reduction in earning power was 30 per cent for 6 months. He estimates the average loss of earning power of an individual infected with malaria to be 25 per cent. Van Dine (1916), who, after a very careful study of the losses from malaria on a plantation in Louisiana, U. S. A., found that each case of the disease caused a loss of time equivalent to 6.42 adult days, also estimated that the loss from inefficient labour was equal to another 10.7 adult working days, *i.e.*, about half as much again as that produced by absence from work due to the attack.

Deeks (1929), on the plantations of the United Fruit Co. in the West Indies, found that, as the health of a district improved with anti-malarial measures, the amount of sugar-cane cut per man per diem rose from 1 ton to 1.61 tons, *i.e.*, an increase of about 60 per cent.

Various workers in India have also made suggestions as to the loss of efficiency caused by malaria in different populations afflicted with this disease.

Watson (1924) studied the labour loss on some tea estates in Assam. He found that, on a total of 10 estates showing an average spleen rate of 69 per cent, the loss from sickness and inefficiency was from 2.4 to 15.4 per cent, with an average of 6.2 per cent. He adds that in a place free from malaria, the inefficiency rate from all causes does not exceed 1 per cent of the working population, or 0.75 per cent of the total population. The Malaria Commission of the League of Nations (1930) say that Ramsay in Assam managed, by

anti-malarial measures, to double the amount of labour done by the same number of coolies.

In tea gardens in the Bengal Duars, Rice (1931) says that 60 per cent of the total sick are incapacitated by malaria, or by other diseases in which malaria has been the primary cause of lowering the resistance of the patient. In his work on the economic loss caused by malaria, he takes 10 per cent as the loss of efficiency due to malaria, and considers this a low estimate. Rice and Savage (1932), in tea estates in Assam, think that the annual loss of efficiency among the labour is about 35 per cent, much of which is due to malaria. Clyde (1931) in the work on the Sarda Canal among a controlled population found that, at the headworks in the Terai of the United Provinces from October 1927 to June 1928, the average monthly loss of man-hours was 10·8 per cent. These Indian figures refer to populations who receive treatment for their attacks, so the loss of efficiency must be considerably less than among untreated populations.

In the evidence given before the Royal Commission on Agriculture in India (1928), one of the features stressed was the marked deterioration of the physical well-being and of the working efficiency of the population that is caused by malaria. For some 3 months in every year the capacity for labour of a large proportion of the inhabitants of rural Bengal, especially west Bengal, is much impaired by attacks of malaria. In Burma, this liability to malaria undoubtedly reduces the working efficiency of a large part of the rural population. In the United Provinces, the loss of efficiency for the 18 million workers in an agricultural population of 35 millions is put down as 50 per cent from malaria. These figures refer to populations which receive little, and usually no, specific treatment for attacks of this disease, and probably not 1 per cent ever receive adequate treatment.

The data available suggest that the loss of efficiency among the malaria-infected persons in an Indian population may be at least as high as 25 per cent. If we consider the 33 million adults who suffer from malaria annually in British India, and take their wage-earning value at an average of Rs. 7½ per mensem, even a 10 per cent loss of efficiency would mean a loss of productive power equivalent to Rs. 2,970 lakhs. If, however, we take the loss at 25 per cent of efficiency, the financial wastage would be Rs. 7,425 lakhs per annum, or about £55 million sterling.

As mentioned above, the figures given by Van Dine (1916) suggest that, among treated patients, the amount of work lost due to inefficiency may be 1½ times that caused by the acute attack. If this estimate be used, the calculated loss due to post-malarial inefficiency may lie between about Rs. 2,000 and Rs. 5,000 lakhs per annum.

Such estimates do not take into account the enormous amount of sickness and disability caused by other diseases to which malaria predisposed the population.

Summary.

Calculations made from minimal figures show a financial loss due to post-malarial inefficiency equivalent to about Rs. 2,970 lakhs per annum, or £22 million sterling. The evidence available suggests, however, that the figure may even be as high as Rs. 7,425 lakhs.

(3) LOSS OF EMPLOYMENT, ETC.

The individual who is constantly suffering from attacks of malaria cannot hope to keep any fixed employment. Apart from the inconvenience, etc., caused to his employer by frequent absences from work, the latter will be loathe to employ or retain the services of one whose labour is not equal to the market standard.

If the sufferer be an agriculturist working on his land, frequent illnesses, especially those during the crop season, will mean that his profits from the land will fall. This in India usually means that he gets into debt, and so a load of poverty is superimposed upon a burden of malarial inefficiency and sickness.

(c) FINANCIAL LOSSES DUE TO DEATHS.

'We have been very careless in the proper valuation of human life. Cattle, hogs and sheep are much more respected than men and women, boys and girls. If foot and mouth disease should break out in a herd of Texas cattle we would spare no expense to stamp out the dread disease that was killing our prize animals. My State, be it said to her shame, spends twice as much protecting the livestock as it does in protecting humanity' (Stanley, 1932).

'It is the cheapness of the slave that made possible the enduring monuments in stone raised by the Pharaohs; it is by leisure at the cost of slave labor that Greek philosopher and Roman poet became the authors of that more lasting structure, classical literature. Thus the price of man has played a rôle of no small importance in history in shaping the world to its present state'. 'Our greatest assets are not our mines, our forests, our rivers and our harbours, but the men and women who control these assets' (Dublin, 'The Money Value of Man', quoted by Stanley, 1932).

Apart from any humanitarian aspects of the subject, it is necessary to consider the financial loss which a death from malaria inflicts upon the family and the community.

Under this heading must be discussed (1) the loss due to unprofitable expenditure on funerals, ceremonies, etc., and (2) the loss of capital due to the value of the lives cut short.

(1) UNPROFITABLE EXPENDITURE ON FUNERALS, ETC.

There is a large unprofitable expenditure for the funeral expenses of the victims of malaria. It might be argued that these people must eventually die, and thus the expenses must be incurred at some time. As malaria has a definite effect in lowering the expectation of life at all ages, and the heaviest mortality occurs in the earlier years of life, the victims of the disease are, in many instances, carried off before they have had an opportunity of contributing little or anything towards the cost of their own funeral expenses. They have not had time to contribute fully either towards the economic prosperity of their country or their own families. The younger they die, the less have they contributed and the greater is the drain on the country for unprofitable expenses.

These expenses include not only the cost of the actual disposal of the body, but also of the funeral ceremonies and the ritual connected with the religion of the deceased. In the case of Mohammedans, proper interment includes the cost of many yards of cloth, the cost of the grave, etc., while with Hindus, the cost of cere-clothes and of wood for cremation are important factors. To these in each case must be added the costs of the different funeral ceremonies.

Colonel W. G. King, I.M.S., in an unpublished memorandum in 1911, calculated that the cost of the funeral with its associated ceremonies in India would average Rs. 12 in the case of an adult, and Rs. 4 in the case of a child. Local enquiries in the Punjab indicate that, at the present time, these estimates cannot be considered excessive.

From the work of different observers (*vide supra*), it appears that about 40 per cent of the malarial mortality occurs among adults and about 60 per cent among individuals under 15 years of age. If, therefore, we accept even one million deaths as being due directly to malaria, and divide these proportionately to age, the unprofitable funeral expenses of the adults would be Rs. 48 lakhs, and for children Rs. 24 lakhs, or a total of Rs. 72 lakhs per annum, on the basis of Colonel King's figures.

If, however, we were to include the deaths due indirectly to malaria the sum would mount up to at least Rs. 100 lakhs.

If one takes the funerals of children only as representative of unprofitable expenditure, in that they have contributed little towards the wealth of either the state or the family, the direct annual loss from malaria is Rs. 24 lakhs. It has been noted above that 43·2 per cent of the infantile mortality in India during the quinquennium 1927—1931 was due to 'infantile debility and malformation including premature birth'. This means that of the recorded infantile mortality of 1,633,476 in 1931, over 700,000 were reported as due to the causes mentioned. The commonest cause, direct and indirect, of these deaths appears to be malaria although they are not registered under that heading. If we consider that even 400,000 of these additional deaths are due to the action of malaria on either the mother or child, we have another Rs. 16 lakhs added to the unprofitable expenditure. We may, therefore, estimate, leaving out any question of deaths among adults who may have earned enough to cover the cost of their own funeral expenses, that the unprofitable loss due to the funerals of children must be at least Rs. 40 lakhs per annum.

It appears reasonable, therefore, to estimate that India loses each year from unprofitable funeral expenses, at least, a sum between Rs. 40 lakhs and Rs. 100 lakhs, or between £300,000 and £750,000 sterling.

(2) WASTAGE OF CAPITAL DUE TO VALUE OF LIVES LOST.

This loss must be considered both from the point of view of loss of revenue to the state and from the economic value of a human life. The former factor will be discussed in another part of this paper.

Attempts have been made in different countries to estimate the value of a human life. It must be remembered, however, that the economic value of a life varies very considerably with age, nationality and local labour conditions, so much so that practically any and every estimate is largely a matter of conjecture.

Balfour (1928) states that Woods and Metzger showed in 1927 that the average *per capita* value of the population of the United States of America was £3,113, while in England, Nankivel, distinctly cautious, says 'it is not an exaggeration to suppose that a life is worth on an average at least £100 to the community'. Mackenzie (1929b) gives the value of a life to be £500. Herms (1913), in estimating the cost of malaria to the State of California, gives the economic value of an adult life as about \$5,000. A similar figure is given for the citizens of the United States by Fuchs (1922), and in a Medical Bulletin from the Missouri

University (1916). 'According to Dr. Dublin, we enter life with a known value ranging from five to fifteen thousand dollars. The money value of the individual grows as we grow in years and in earning power' (Stanley, 1932).

In India, Kunhardt (1919) calculated the value of a life at that time to be Rs. 600. In the tea gardens of the Bengal Duars, Rice (1931) estimated that it cost Rs. 300 to obtain a coolie for the estates*. Rice and Savage (1932) state that the value *to an estate* of a settled working coolie is Rs. 400 in the Duars, and Rs. 500 in Assam.

Before one can make any estimate of the loss to India caused by deaths due to malaria, it is necessary to consider what is the average annual income per head of the population, and the wage-earning power of an adult. This subject has been dealt with in discussing the value of the days lost due to malarial sickness.

As mentioned previously, the average expectation of life in India at birth is about 23 years, at the age of 20 years about 27, and at the age of 50 about 14 years. It appears, therefore, to be justifiable to take 20 years as the average loss of life of each individual who dies of malaria.

From these data, it is possible to make some estimations as to the financial loss incurred by India from deaths due to malaria.

(i) Boag (1916) concludes that the most satisfactory measure in calculating the value of a human life is the capitalisation of a person's total income. This means that each death from malaria is equivalent to an average loss of 20 years of income at Rs. 45 per annum, or Rs. 900. The million deaths yearly from this disease would, therefore, mean a loss of Rs. 9,000 lakhs, or £67½ million sterling, by this method of estimation.

(ii) It is probable (*vide supra*) that, at least, 30 per cent of the deaths directly due to malaria occur between the ages of 15 and 50 years, which for the purposes of calculation will be called the wage-earning age period†. The census returns indicate that between these ages the sexes are about equally represented. The data already given show that in this age period probably at least 150,000 males, and the same number of females, die from malaria each year. The value of the work of an adult male may be taken at Rs. 10 per mensem, and of an adult female at Rs. 5, or an average of Rs. 7½ for both, as the two sexes are about equal in number‡.

From these figures, the loss may be calculated in another way, the value of a human life may be established as the lump sum which must be paid to an assurance company to obtain the income of the individual for half the working period of life, *i.e.*, for 17 years.

At the average age of 32 years, the sum which will provide an annuity of Rs. 90 for 17 years is Rs. 1,500. The loss of 300,000 adult lives would be worth

* These figures only represent the cost of recruiting and equipping a coolie, and *not* the capital sum which his work is worth.

† In India, large numbers of the population earn wages or their equivalent, both before and after this age period. Again as in previous calculations we have taken the minimal figures as the basis of our estimate.

‡ The heaviest malarial mortality falls upon the rural population who form 90 per cent of the people of India. Of the total population 80 per cent are engaged in agricultural occupations. Under these conditions both the males and the females work in the fields, so the wage-earning value of both sexes must be included.

on this basis Rs. 4,500 lakhs, or £34 million sterling. This estimation does not take into account the potential and actual value of the other 700,000 individuals who also die of the disease each year*.

(iii) The *Hindustan Times* (Delhi) in its issue of 23rd September, 1934, gives the comparative income *per capita* of persons in the United States of America as Rs. 1,070, in Great Britain as Rs. 750 and in India as Rs. 45. If we take the value of a life to be in corresponding ratios in the different countries, that of one in India may be calculated from these figures. The value of a life in the United States is estimated at \$5,000, and on this scale one in India would be worth about Rs. 600, which is what Kunhardt (1919) calculated sixteen years ago. If the value of a life in Great Britain is £500 then one in India would be worth Rs. 400 approximately. When calculated from these figures, the loss due to a million deaths each year from malaria would lie between Rs. 4,000 lakhs and Rs. 6,000 lakhs per annum.

In all the above calculations no consideration has been taken of deaths indirectly due to malaria. If these be taken into account the figures would probably be doubled.

Summary.

From the data at our disposal, it appears probable that the economic loss to India from deaths directly due to malaria is, at least, Rs. 4,500 lakhs per annum, but is more probably nearer, if not more than, Rs. 9,000 lakhs, or about £67½ million sterling. This sum does not take into account the fact that probably an equal number of persons die each year from the indirect effects of this disease. As was pointed out when speaking of the relationship between malaria and economic stress (p. 252), the death of a wage-earner may result in an enhanced mortality among, or even a complete obliteration of, the family afflicted.

(d) MISCELLANEOUS EXPENSES TO THE INDIVIDUAL OR FAMILY.

Families living in malarious areas may need to send away their wives and children during the period of the year when the disease is most prevalent. This necessitates the expense of the payment of two rents, apart from the money required for travelling and for the upkeep of two different establishments. To this must be added the cost of sick leave for members of the family who fall ill, if they can afford such an expense.

If the family has acquired property in a malarious area, excessive sickness may cause them to abandon it. This means a loss of much of the value of the holding, for there may be difficulty in finding a buyer, loss of time, money and energy spent in developing the property before it had to be abandoned.

(e) CONCLUSIONS.

Although it is impossible to obtain any accurate figures as to the financial loss caused to the individual or the family each year by the ravages of malaria, there is very much good evidence to suggest that this cannot be less than

* The large number of deaths among children represents a great loss of capital to the family and the state. Much money has been invested in the upbringing and education of these children, from which no return can be expected until they grow up. If they die during their early years, there is a complete, or almost complete, loss of the capital invested.

Rs. 10,760 lakhs, or about £80 million sterling. It is even probable that the amount may be twice this sum at least.

(III) FINANCIAL LOSSES TO THE COMMUNITY, DIRECTLY AND INDIRECTLY.

'The reduction of individual earning and spending capacity, of agricultural and industrial production, affects the prosperity of the entire community—the merchant, the landowner, the railroad. Where malaria seriously prevails it does more injury to the community than any other disease; indeed, more than all others combined. No such community is, or can be, prosperous' 'The market value of land, whether for suburban homes, summer resorts, manufacturing sites, or farms, remains low. Industries are not established, agricultural settlements are not made, farms are abandoned, and enterprising persons move to a more favourable locality' (Fuchs, 1922).

'Other diseases cause a notable loss which is prompt and easy to see. Malaria causes a gradual insidious loss difficult to figure but which affects all phases of the community life' (Johnson, 1926).

'Nothing costs the individual ratepayer more than sickness and mortality' (Nankivel, quoted by Balfour, 1928).

The loss which malaria causes falls upon the common people, the landlord, the merchant, the planter, the industrialist and the banker as well as upon Government. These are the main classes of individuals involved in the economic loss to the community. 'The losses from malaria form a vicious cycle each depending on the other. These indirect losses are far the largest losses from the malaria problem; they result in making the community an expensive one to live in and render it unattractive to outside enterprises looking for new locations' (Johnson, 1926).

'The common people and the labouring class in general are direct losers in the way of time lost, doctors' bills, medicine, and lowered vitality. They also suffer an indirect loss due to the poorer economic condition of the community. The direct loss is by far the smallest caused by malaria' (Johnson, 1926).

A community afflicted with malaria has, as a whole, a reduced earning and productive power. The cost of living is raised and the necessities of life are difficult and expensive to obtain. The income of the individual is less, and his spending power proportionately diminished, so a vicious cycle arises whereby 'malaria begets poverty and poverty malaria'. In India a burden of debt is often accumulated under these circumstances, and this helps to make the condition even more serious (*vide* 'Economic Stress', p. 252). The more enterprising and capable individuals tend to leave the area, conditions go from bad to worse, so that 'the inhabitants of a malarious district tend to become a mere residue of the poor and wretched'. The material and social 'bonification' of the community, which follows upon anti-malarial measures, tends of itself to give rise to a continued diminution of the incidence and severity of malaria (*vide* Sinton, 1933*).

* 'With this condition of "bonification", most of the adverse influences which acted as causal factors of high malarial incidence have disappeared. The people are well housed in habitations less attractive for the shelter of mosquitoes. Overcrowding has diminished. Screening has been attempted in some dwellings. Over-work and the scarcity of good food have largely disappeared. Domestic animals are plentiful and are housed under conditions which attract mosquitoes for shelter. The sick can afford to avail themselves of prompt and effective treatment. With the natural increase of the population, the demand for immigrant labour diminishes with a resultant decrease in the introduction of infection'.

Under conditions where a severe epidemic of malaria occurs, as happened in the Punjab and parts of the United Provinces in 1908, the financial losses to the community must be very heavy. Christophers (1910) states that in Amritsar, for example, for many weeks the ordinary business of the city was interrupted, labour could not be obtained, transport was disorganised, and even food vendors ceased to carry out their duties. Mackenzie (1923) records a similar state of affairs in the epidemic in South Russia.

The damage which malaria causes to the value of the property of landowners in the community is marked. The market value of land in malarious areas remains low. There is little or no demand for such land for building purposes, because prospective buyers will be kept away by the unenviable reputation of the area for unhealthiness. Agriculturists will not take up farms there, even when the land is good, the price cheap and the rent low, because of the difficulty and expense of obtaining effective labour. Manufacturers will not acquire sites for industries for the same reasons. Such employers of labour will find it difficult or impossible to compete with the rates and output of their rivals in more healthy areas.

These circumstances, especially if there be any further increase in malarial incidence due to the introduction of imported labour, often lead to the abandonment or forced sales of such agricultural, industrial or mining concerns. This means a loss in rent or profit to the landowners, and a further depreciation in land values, apart from the loss incurred by the individuals or companies who have had to abandon their undertakings. All these facts react adversely upon the welfare and prosperity of the community.

'The merchant's loss is indirect. He is operating in a place where funds are diverted on account of sickness; money is scarce on account of inefficient labour; he sells less, therefore he must have higher prices, and he is working under a depressed market condition' (Johnson, 1926).

'The banker's loss is indirect. Less money is available; the demand for it is greater; the risk of the banker is larger. Short term loans under rigid requirements as to payment are the rule, which in turn tend to limit or curtail desired business expansion' (Johnson, 1926).

The employer of labour—the agriculturist, the industrialist, the mining company, the shipper, the railway, etc.—lose both directly and indirectly in malarious areas. Labour is often difficult to recruit, and usually expensive, as compared with more healthy areas; its efficiency soon becomes low, and its output is often smallest at most important periods of the work. This results in a curtailed production, increased overhead expenditure and lost markets, for in many industries the cost of labour forms 40 to 50 per cent of the cost of production. The action of malaria upon such commercial enterprises is so damaging, and affects so adversely, not only the prosperity of the local community but the nation as a whole, that they will be discussed in more detail in the next sections.

The taxes of the community for administration and other purposes, are higher in proportion to their income, than those of more healthy and prosperous localities. The community feels the burden more. The lowered efficiency, both mental and physical, of the officials employed, means that the community also gets a poorer return for the money expended.

The absence of children from school on account of malarial sickness means that the standard of education in the community as a whole is lower than that of more fortunate localities.

All these factors combine to produce a vicious cycle and to militate against the physical and mental health, and material prosperity of the community. The latter has to bear these, in addition to the heavy losses which have previously been discussed as falling upon the individuals and families which form the community. These indirect losses cannot be estimated in rupees, annas and pies, but, when their combined effects are considered, the financial wastage is almost certainly much heavier than that caused by the direct effects of the disease.

It is almost impossible to make any estimate of the loss to the community caused by malaria. Bentley (1911*b*) estimated the loss of the City of Bombay at nearly Rs. 30 lakhs during the year 1908.

Bentley (1911*b*) calculated the loss from sickness *alone* was at a low estimate Rs. 12 to Rs. 13 lakhs per annum. 'The figure given above does not include loss of rent to landlords caused by the abandonment of houses in malarious localities and the loss of tenants who are forced to leave and perhaps pay two rents for a time; it makes no allowance for loss to private persons forced to go to considerable expenses for hospital attendance or perhaps compelled to resign their appointments and leave the City to recover their health; and it does not include the loss occasioned by the disorganisation and lessened efficiency of a business staff, a considerable proportion of whom are from time to time disabled by sickness'. 'If these and other losses, occasioned to the citizens of Bombay by malaria, could be totalled up, in all probability it would be found that the sum of Re. 1/4 to Re. 1/8 per head of the population, which has been taken at an estimate, represents only one-half or one-third of the amount paid away without return each year by the citizens of Bombay because of an easily preventable disease in their city'.

Covell (1928) considers he is 'justified in estimating the average annual cost of malaria to Bombay at present as not less than 50 lakhs of rupees'. This estimate of Covell's is equivalent to a loss of almost Rs. 5 per head of the population per annum. If we consider the average earning capacity of each member of the total population of India to be only one-fifth that of an urban inhabitant in Bombay, the loss to India would be about Rs. 3,530 lakhs per annum, or £26 million sterling. This loss is due to sickness *only*, and does not take into account the probably even greater loss due to the retardation of development and commercial progress.

(IV) FINANCIAL LOSSES TO AGRICULTURE, INDUSTRY AND COMMERCE.

'But malarial fever is important, not only because of the misery which it inflicts on mankind, but because of the serious opposition which it has always given to the march of civilisation in the tropics. It is therefore the principal and gigantic ally of barbarism. No wild deserts, no savage races, no geographical difficulties have proved so inimical to civilisation as this disease' (Ross, 1905).

'Malaria has been one of the greatest foes of civilisation; its operations for evil have continued from century to century. Gigantic commercial enterprises, undertaken at different times, have been abandoned on account of the terrible havoc wrought by malaria. All malarial countries are seriously handicapped, and their natural development towards the highest economic, industrial and political efficiency is materially retarded by their malaria' (Hehir, 1927).

'Malaria affects every race in the tropics and sub-tropics. It hinders development of all tropical countries, and raises the cost of every article produced. As these products are consumed or manufactured in other parts of the Empire, malaria thus raises the cost of

production, hinders the expansion of the markets and raises the cost of living in every part of the Empire' (Annual Report of the Ross Institute and Hospital for Tropical Diseases for 1931).

(a) GENERAL CONSIDERATIONS.

So far we have dealt only with the disease from the point of view of the person affected, or his family or community. There is, however, an outlook which may be called the economic, in which Government especially may be expected to be concerned, and another view, the financial, in which both Government and various other bodies are concerned.

'The effect of malaria on commercial enterprise and the economic loss to the community caused by malaria have never been really satisfactorily worked out. The economic loss caused by malaria to the individual, the community and to any commercial enterprise are incalculable. Besides loss through untilled acres, diminishing earning capacity, loss of time, and death, malaria produces in its victims a disinclination for work whose influence cannot be estimated in money' (Phillips, 1929).

'A point of some importance is what a country pays for a bad sanitary reputation. Such a country is apt to be mulcted by health restrictions, sanitary regulations and quarantine applied to its shipping and ports, all entailing delay to commerce and perhaps even directing trade elsewhere. Shipping firms have to maintain agents and to pay them in accordance with the sanitary reputation of the country. Firms have to do the same. All must ultimately be paid for by increased cost of necessities to the country concerned. A recent bulletin gives the value of India's annual imports as over 200 crores of rupees and that of her exports as about 300 crores. A very small *ad valorem* percentage as a result of such influences as we have indicated would very soon run into crores. This is a modest statement of the case against disease in India. The cost I cannot pretend to put into figures. All I can say is that the tribute paid to disease in a country like India is one of importance economically, even politically, and one that has many financial and commercial aspects. It, however, transcends this in being of importance to the welfare of 360 million human beings' (Christophers, 1924). While the above statement is made about the losses which disease generally causes to India, the one which has the greatest influence in producing these losses is undoubtedly malaria.

The financial and economic losses which malaria causes to any country or industry are all, directly or indirectly, bound up with its effects upon the health and working capacity of the individual, either singly or combined as an organised labour force. Watson (1921) says that in Malaya the labour problem is identical with the malaria problem, and this dictum is true of almost all countries where the disease is prevalent.

As has been noted previously, the individual infected with malaria is less efficient and able, both mentally and physically, than is a healthy person. The employer who engages such a worker will not only obtain a relatively poor return for the wages paid, but will also be liable to lose the services of the labourer at frequent intervals, often when these are most needed. The presence of malaria in a locality often means that there is a deficiency of available or suitable labour. This may be caused by (i) the sparse population which malaria leaves in many such areas, or (ii) a high rate of morbidity among the

local population, or (iii) the high wages required by immigrant labour and the difficulty in retaining the services of such labour.

In malarious districts, the disease may strike a labour force at some critical period of the operations, and so result in great financial loss. Outbreaks of malaria of appalling severity are liable to occur under tropical conditions, where large masses of labourers are aggregated together. These conditions are especially liable to occur during the course of large operations, involving changes in the configuration of the ground, such as the construction of railways, roads, docks, harbours, canals, bridges, etc.

The disastrous effects of malarial sickness upon labour forces have been felt by almost every branch of human activity in the tropics. These effects cause not only large financial losses in the initiation of large undertakings, but delay in their completion. When finished the operating costs of such enterprises are usually much higher than those in healthier localities.

Apart from its direct effects on labour of all kinds, the higher cost of working and the difficulties which this disease places in the way of successful operations, cause an incalculable loss to the development and prosperity of any community and country where it seriously prevails.

The general aspects of the labour problem in relation to the individual and the family have already been dealt with. It is, however, necessary to discuss the special action of malaria upon various kinds of industrial, commercial and agricultural activity in India.

(b) THE LOSSES WHICH MALARIA CAUSES TO AGRICULTURE.

'In man's history food may be claimed to have been of immensely greater importance than climate' (Osborne, 1920).

'Malaria is a disease of waste water, waste land and waste people' (Perry, 1914).

'Neglect of agriculture may undoubtedly tend to increase malaria, and it is of course impossible to separate definitely the influence of malaria upon decay from that of decay upon malaria'. . . . 'Malarious regions are generally extremely fertile'. . . . 'The increase of malaria is an economic calamity which robs a country of its most precious source of wealth. So tempting is the chance to become rich that many come from more healthy quarters, and, with their lives in their hands, endeavour to reclaim the land which has been abandoned by their predecessors'. . . . 'But in spite of these often repeated attempts, rarely permanently successful without the help of modern science to work profitably on malarious sites, the economic loss is enormous' (Jones, 1909).

'To the farmers it may mean the loss of crops from want of cultivation. It will always mean the non-cultivation or imperfect cultivation of thousands of acres of valuable land' (Howard, 1909).

'Unhealthy (malarious) lands can only be cultivated at the risk of the life of the worker, and the substance of the owner' (Varro, B.C. 118, quoted by Bentley, 1908).

As pointed out by Hehir (1927), 'compared with agriculture, the other industries of India, though they play a useful part in the economic life of the country, are of minor importance. Agriculture is, and always will be, the great field of industrial activity, for upon it the welfare and prosperity of the large masses of India depend'.

That malaria is the great enemy of agricultural prosperity in the tropics and sub-tropics has been recognised in many countries outside India.

In speaking of Italy, Celli (1904) states that 'if we calculate, owing to malaria, about 5,000,000 acres of land and very many localities, as for instance the Agro Romano, remain uncultivated, the economic loss from it must be enormous'. He also points out that many territories are uninhabitable because of this scourge, and sums up the situation by saying that 'malaria costs Italy incalculable treasure'.

Many workers in the United States of America have drawn attention to the loss which malaria causes to agriculture, more especially in the Southern States. Howard (1909) says 'the loss to this country in the way of retardation of the development of certain regions, owing to the presence of malaria, is extremely great. Certain territory containing the most fertile soil and capable of the highest agricultural productiveness is practically abandoned'. . . . 'These regions in the absence of malaria would have added millions to the wealth of the country'. Fuchs (1922) and several other American authors have made similar statements. Carter (1922) has compared the fertility of the land in the Southern States with that in the Northern. He says that while there is as good land in the former as in the latter, the land in the north sells at about 12 to 20 times the price, the difference being mainly due to malaria. The same worker is quoted by Fuchs (1922) as saying:— 'On the peninsula of Virginia the land is good, markets convenient, cultivated communities close and easily accessible, and agriculture in Virginia as a rule is prosperous; but people can not work and will not live where malaria seriously and continuously prevails, and they gradually abandon the section'. 'The loss of value of these lands he hesitates to estimate— certainly over \$30 per acre'.

Fuchs (1922) reports that a deal involving the purchase of a half-million dollar tract in eastern North Carolina for agricultural colonisation was never consummated on account of the presence of malaria. Bass (1918) says that if any Delta county could eradicate malaria so as to be able truthfully to say to the world that there is no malaria there, the value of the land would be doubled in a short time.

In an official handbook issued at the British Empire Exhibition at London in 1924, malaria is given as 'one of the chief reasons why such large malarious tracts of fertile land in Malaya remain sparsely populated and undeveloped'.

Malaria has an inimical effect upon the development of all types of agriculture in India. As about 80 per cent of the population of India are employed in agricultural work, and 90 per cent inhabit rural areas*, the prevalence of this disease is of the greatest importance to the prosperity of the country.

It is, however, the small farming class and the agricultural labourer rather than the employees of plantations, mines, etc., who have to bear the brunt of the malarial sickness in India, and its effects. The larger commercial enterprises who employ labour very often realise that it is a paying proposition to take steps whereby such sickness is diminished among their staff and employees. In the case of the rural masses, however, 'here are no capitalised communities of pioneer modern spirits imbued with a progressive modern outlook—familiar with, and ready to utilise, the most recent forms of machinery and scientific invention. On the contrary, in the vast bulk of population is the conservative spirit of those whose ancestors have always lived as they have—communities whose combined resources are woefully small—so poor that they practically cannot be taxed in the western sense, lacking in public spirit, with a tremendous amount of ignorance and apathy, having no "health conscience" or even any adequate appreciation of what is in the mind of the sanitarian who may attempt to rouse their enthusiasm in such cause' (Christophers, 1930).

'Cities being, as a rule, less malarious than cultivated plains, the urban population tends to absorb the agricultural classes, and the national physique and well-being suffer in consequence' (Jones, 1909). The Public Health Commissioner with the Government of India in his Annual Report for 1931 gives figures for the urban and rural death rates from malaria in different

* 'The main occupation of the country people, of whom barely 7·8 per cent can read or write, is agriculture and stock keeping. In British India, 125 million small farmers with their families (73 per cent of the population) get their living out of the soil, while the rest of the rural population is employed on the large plantations, in the mines, in public works, trade, etc.' (Malaria Commission, League of Nations, 1930).

provinces in India. The rural rates in Bengal were 9.2 times the urban ones, and those of the United Provinces about 4 times. If we take the rural population of nearly 109 millions in Bengal, the United Provinces and Madras combined, the total death rate is 11.8 per mille, as compared with 2.4 per mille among the 11½ millions of urban residents, i.e., the death rate in rural areas is nearly 5 times as heavy as that in urban ones, and total deaths about 50 times as numerous. Banerjee (1916) says that out of 965,000 deaths registered from 'fevers' in Bengal in 1913, only 25,000 were from urban ones, the rest being among the rural population. These figures suggest that about 40 to 50 times as many 'fever' deaths occur in rural areas as in urban ones. As malaria is pre-eminently a rural disease, and agriculture is the chief support of the country, the loss which the staple industry of India sustains must be enormous. The influence of this disease upon agriculture is therefore one of the most important economic problems of India*.

In discussing the economic losses which malaria causes to the different types of agriculture in India, the following special points may be considered :—

- (1) The effects of malaria in retarding agricultural development.
- (2) The effects of malaria in causing the abandonment and decline of agriculture, and
- (3) Special labour factors in relation to agricultural development.

(1) EFFECTS OF MALARIA IN RETARDING AGRICULTURAL DEVELOPMENT.

The effects of malaria in hindering and retarding the progress of agricultural development in different parts of India have been noted by many workers.

Bentley (1925) has made a special study of the relationship between agriculture and malaria in Bengal†. He states that as a broad fact only 58 and 60 per cent of the cultivable area in Central and Western Bengal, respectively, are under cultivation, while in the much less malarious eastern portion (*vide* pp. 228-229) the percentage is 90. He considers that the relative prevalence of malaria in Bengal is largely influenced by the same conditions which determine a good or bad harvest.

Government has for many years attempted to colonise the Terai of the United Provinces with immigrants from other parts of the plains. As pointed out by Robertson (1909), the soil is good and fertile, and well repays cultivation‡. After describing the awful malarial state developed by these new-comers, the latter worker considers that 'any attempt at reclamation under present (malarious) conditions, however, can never hope to be successful, as they are dependent on a constant immigration to keep up the population'. Phillips

* 'Further, 90 per cent of this population is living under rural conditions. This vast illiterate teeming mass of humanity, living in 687,935 towns and villages, is the real malaria problem of India in its ultimate and complete aspect' (Christophers, 1930).

† The question of the relationship of malaria to agriculture in Bengal has been dealt with in great detail by Bentley (1925) in his book 'Malaria and Agriculture in Bengal'. All workers interested in this subject, more especially in relation to Bengal, should study this work carefully.

‡ 'There exists along the foot of the Himalayas in these parts (the Terai) a broad belt of grass land, many hundreds of square miles in extent, almost uninhabited and entirely unexploited. The soil and other conditions agriculturally were said to be favourable and the whole question of the commercial value of utilising the land could be seen to turn on the prevalence of malaria' (Christophers, 1924).

(1925) also studied a portion of this area, and says 'within recent times malaria has worked such havoc among the inhabitants of this tehsil (Gadarpur) that through ill health, diminished adult population and general inability on the part of the people to carry out their responsibilities as agriculturists, the revenue has fallen considerably'. He reports the steady decrease in population which has occurred since 1881 (*vide* p. 230), and goes on to remark that 'with rent and taxes low and with the financial aid given by the Estates in the construction of villages and huts the people ought to be, and are prosperous. But the general unhealthiness of the climate and the distressing and permanent incapacity caused by malaria produced a *Desa* (immigrant) population, at any rate of poor physique, lethargic and unfit'. On the other hand, the indigenous aboriginal people are much more robust and healthy.

Perry (1914), in his malaria survey of the Jeypore Hill Tracts of the Madras Agency, points out that 'much of the country with its abundant rainfall is of very great agricultural value'. On account of its malarious character, it is now but sparsely inhabited by people mostly of aboriginal origin—'the truth almost certainly must be that were it not for the great virulence of the malaria which these people harbour, and the ample anopheline fauna of their jungles, such tribes would have been absorbed into the great Dravidian and Aryan civilisations of India, and practically lost sight of long ago'. Of the highly malarious lower plateaus of this tract, Perry (1914) says that, from its physical character and nature, 'this is a country.....which has rightly been described as one "crying out for development"'.

Rao (1929a) says that the officers attending the Madras Agency Conference in 1927 informed Government that 'the prevention of malaria in the Agency Tracts was by far the most important question to be dealt with, and that until effective steps had been taken to grapple with this problem, it would be a matter of the greatest difficulty to procure the necessary labour for the improvement of communications by road and railway or to secure the population required to develop the important agricultural and forest resources of these tracts'.

Kenrick (1914) points out that, in one area in the Central Provinces which Government was trying to reclaim, it was found that 'malaria offers a serious obstacle to successful colonisation. The soil is good, subsoil water level high, and a very low rent-rate demand, but settlers, especially those from the plains, complain of the severe type of fever prevalent'.

These data give some indication of the hindrance to agricultural development which malaria places in the way of the successful exploitation of large and valuable tracts of very fertile land by the rural population of India. While this aspect of the problem is the most important one to India as a nation, one must also consider the obstacles which this disease places in the way of large commercial undertakings.

The cultivation of the products grown by many of these large companies (tea, coffee, rubber, etc.) often require special climatic conditions for their successful development, but unfortunately these conditions are very frequently those which are also favourable to a high malarial incidence. As pointed out, malarial sickness by its effects upon the staff and labour force of the plantations, gives rise to a large amount of inefficiency, etc., and is probably the chief element in retarding the development of such undertakings and in causing heavy financial losses.

The effects of malaria in producing inefficient and deficient labour forces have been felt by the tea industry in most parts of India where it has been developed. The action of this disease has added enormously to the cost of production on the estates of Bengal, Assam and Madras. This is pointed out in the reports of Bentley (1908), Christophers and Bentley (1909), Watson (1924), Sur (1926), Ramsay (1927, 1930a, 1930b), Macdonald and Chowdhury (1931), Manson (1931), Manson and Ramsay (1932, 1933), Strickland *et al.* (1928, 1929), Horne (1914), Ross, T. S. (1914), Rao (1915), Clemesha (1931), Clemesha and Moore (1930) and many other workers.

Apart from the tea industry, the development of plantations of coffee, rubber, pepper, cardamoms, etc., on the hills of Southern Madras (Wynaad Plateau, Nelliampatti Hills, Anaimalai Hills, Palni Hills, etc.), have been seriously hampered by the ravages of malaria (Horne, 1914; Ross, T. S., 1914; Rao, 1915; Rao, 1929a, 1929d). In the plantations in the Anaimalais, Ross, T. S. (1914), has drawn a special attention to this damage.

'At first the planters were not hampered by malaria amongst the coolies but as more labour was introduced—much of which coming from plantations in other parts was already malaria-infected—the disease gradually increased until at present it assumes in certain seasons such proportions as seriously to affect the supply and fresh recruiting of labour. It is therefore already of considerable economic importance and if allowed to continue unchecked a time will very soon arrive when it will be impossible to find sufficient labour for all the estates opened and about to be opened on these hills'.

The sugar industry in several parts of India has also been damaged by the effects of malaria upon its labour forces. Hodson and Akula (1921) were called upon to investigate the high incidence of malaria among the labourers at the Belapur Sugar Estate, Bombay.

Christophers (1925a) examined the malaria problem on the Nalbari Sugar Estates in Assam. He states that 'it is an experimental venture in which Government takes a considerable interest in view of the fact that the land on which the Estate has been opened is representative of a large tract of country suitable for sugar cultivation, but at present practically uninhabited and unexploited'..... 'The soil and other conditions are said to be favourable and the whole question of the commercial value of utilising the land would seem to turn upon the prevalence of malaria'..... 'Those working on the Estate clearly indicate malaria as their greatest handicap and as the one condition that may militate against success commercially'.

Malaria was having such a serious effect on the labour at the Sahmaw Sugar Estate and Factory in Burma, that Feegrade (1928), who was asked to report, says 'the high (malarial) morbidity must entail great loss of labour and consequently a large but avoidable expenditure of money'.

These are a few examples of the harmful effects which this disease causes to some of the principal agricultural undertakings financed by commercial interests in India. Very many of these large companies have now realised the importance of malaria as an economic factor adversely affecting their labour forces and so their profits. A number of these concerns are now spending large sums of money in controlling or ameliorating the ravages of this disease. The money so spent is considered to be a sound investment from a commercial, apart from any humanitarian, point of view.

* Since then this enterprise has had to be abandoned on account of malaria.

Along with agriculture may be considered the effects of malaria upon the preservation, development and exploitation of the vast forest resources of India. This matter concerns not only commercial undertakings, but also the Forest Department of Government. The personnel and labour forces of the latter department, in certain areas of the Madras Presidency (*e.g.*, Sapal Hill, Malabar district, and Mount Stuart, Coimbatore district), were markedly affected by this disease (Iyer, 1924; Cunningham, 1925; Menon, 1925*b*). This caused a serious impediment to the proper working of these forests by Government.

Covell (1927) reports upon the severe malaria among the labourers doing forestry work in the Andaman Islands. He records the case of a body of Karen labourers engaged to fell timber, among whom such a virulent outbreak of malaria occurred that the work had to be abandoned. 'The difficulties of the Forest Department with regard to the prevention of malaria are very great'. Phillips (1929) published a bulletin on malaria prevention for the use of Forest Officers. In this he points the grave risks from malaria run by workers in the Forestry Department, and the importance of taking preventive measures against it. Gupta, Das and Majumdar (1932) have written a report upon the prevalence of malaria in the large Government forestry settlement at Kachugaon, in Assam. These workers consider that annual loss in wages, cost of medical aid, drugs, sick leave, etc., works out at about Rs. 10 per head of the population per annum, or about 3 per cent of their total income. This is apart from the loss of Government due to an inefficient and deficient labour force, loss of development, etc. While speaking of the hindrance which malaria places upon the development of the mining resources of Singhbhum, Christophers (1925*b*) also points out the very important forest resources which occur in the same malarious locality.

From the data quoted above it is evident how serious is the obstacle which malaria places in the way of the development of large and potentially rich areas of fertile land. If such land were cultivated or otherwise exploited, it would add enormously to the wealth of the country and would also form an outlet for a very considerable number of the huge population of India.

The economic loss from this retarded development must be very great, or one might express it like Celli by saying that malaria costs India incalculable treasure. It is not possible to make any estimation of the amount of this huge loss.

(2) EFFECTS OF MALARIA IN CAUSING THE ABANDONMENT OR DECLINE OF AGRICULTURE.

Apart from any question of the opening-up of new areas for agriculture, there is the unfortunate fact that many tracts of fertile land, where agricultural enterprises have been started in the past, have now passed wholly out of cultivation, mainly on account of the ravages of malaria upon settlers.

With the advent of malaria, many of the cultivated regions of ancient Greece and Rome are said to have become waste (Jones, 1909; Oliver, 1929; Celli, 1933). Howard (1909) notes that 'it has been suggested that the depopulation of the once thickly settled Roman Campagna was due to the sudden introduction of malaria by the mercenaries of Sylla and Marius'.

The Ross Institute for Tropical Diseases, in its Annual Report for 1931, points out that more than half Ceylon has been decimated by malaria. The extensive ruins of buried cities

and the great irrigation system of tanks show that it once held a dense and prosperous population. To-day this half of Ceylon has a population of only about 40 per square mile of malaria-stricken people. On the contrary, the other half of the island has a population of nearly 400 per square mile.

'In quite recent years malaria entered and devastated the islands of Mauritius and Reunion, practically destroying for a time the productiveness of these rich colonies of Great Britain and France' (Howard, 1909). Mauritius had very large sugar plantations, mainly run by Indian labour, and, as remarked by Balfour and Scott (1924), this visitation wellnigh ruined it irretrievably.

India is not without her record of areas in which malaria has played a devastating rôle in relationship to the development of agriculture. In the Central Provinces, Kenrick (1911) reports upon the depopulation which malaria has caused in certain areas of these provinces, and states that much cultivated land has been replaced by scrub jungle on this account. The same author (Kenrick, 1914) tells how 'in some cases malarious villages with good land were taken up by two or three families in succession, only a few months sufficing for the malaria to clear away each whole family'.

The abortive attempts to colonise the fertile lands of the Terai of the United Provinces have already been mentioned. Clyde (1931) remarks that, if it were not for fresh immigration into these areas, depopulation would occur automatically. He concludes that 'any attempts at reclamation under the present (malarious) conditions, however, can never hope to be successful, as they are dependent on a constant immigration to keep up the population'.

The effects of malaria in causing a decline of agricultural prosperity are especially evident in Bengal. Banerjee (1916) states 'it would be no exaggeration to say that some of the fairest parts of my loved Province have been decimated by this terrible scourge'..... 'I have with my own eyes seen villages once the abode of health, happiness and plenty, now a crumbling mass of dilapidated structures, overgrown with jungle, with a solitary inhabitant here and there, pale anæmic, suffering from malarial fever, but resolutely clinging to the ancestral homestead, as if mounting guard over the sweet reminiscences of the past. Some of our healthiest districts have suffered grievously from malaria. Birbhum is a well known district in Bengal. At one time it was a health resort; it used to be so within my recollection. To-day the health conditions of the place have completely changed. The Sanitary Commissioner in his report for 1914 says it tops the grim list of recorded mortality from malarial fever in the whole province. Take another district, the district of Nadia'..... 'At one time it was a health resort'..... 'To-day it is a plague spot, malarial fever counting its victims by thousands and tens of thousands'. An Editorial in the *Indian Medical Gazette* (1922) states that 'in West and Central Bengal cultivable land has passed out of cultivation, jungle has replaced crops, anopheline mosquitoes have increased, economic conditions have become more severe, and malaria become rife'.

The relationship between agricultural decline in Bengal and the ravages of malaria has been especially studied by Bentley (1925). He gives the histories of several areas, formerly healthy and prosperous, but now highly malarious. The decay of Kassimbazar started from the beginning of the 19th century, before which it was celebrated for its salubrity. It was both exceedingly well cultivated and very populous. Burdwan, Birbhum, Midnapur and Hooghly in Western Bengal were at one time free from malaria and regarded as sanatoria. Similarly Murshidabad and Nadia and greater parts of Jessore in Central

Bengal; Pabna and Malda in North Bengal were also relatively salubrious. In Eastern Bengal, Tangail in Mymensingh, Manikganj in Dacca, and the northern parts of Faridpur are now malarious, but were previously relatively free from the disease. 'The embanking of the country and the shutting out of river water from the surface of the delta was further marked by the simultaneous occurrence of appalling epidemics of malaria, a serious decline of agriculture and the progressive depopulation of the affected areas' (Bentley, 1925). Almost a quarter of a century ago, the *Statesman* in a leader dated 31st January, 1911, also commented upon this havoc :—

'In Jessore district, to take one example, villages are being depopulated by malaria, and by diseases to which it leaves the people an enfeebled prey. No outcry is raised over this mortality. The villagers bow their heads to fate. Death after death occurs, and as the thin ranks of fever-stricken folk who remain are too feeble to keep the village economy in order, its affairs go from bad to worse, until what was once a thriving little community is all but blotted out. No one needs to be convinced that, if this devastation of rural Bengal can be prevented, any practical measures should be promptly adopted'.

A similar decadence of agriculture is noted by Bentley (1925) in other parts of the country—'India, too, can show examples of once prosperous delta areas sinking into a state of decay through agricultural decline and epidemic malaria, and then being restored again to health and prosperity by the reorganisation of their river systems. The Tanjore and the Rajahmundry districts in Madras, which have both passed through these experiences within the last century, are striking instances'.

The effects of water-logging of the soil, with resultant malaria and diminished agricultural prosperity, have been known for many years. The unhealthy (malarious) condition of water-logged tracts in India was recognised by Government nearly 100 years ago, when they appointed a Committee composed of Baker, Dempster and Yule (1847) to enquire into the causes of this unfortunate state of affairs along the courses of the Western and Eastern Jumna Canals. It was during this enquiry that Dempster (1848) introduced the use of the spleen rate, as a means of evaluating the incidence of malaria in different localities.

'In the great Canal Colonies a serious menace is the malaria that is normally induced as the result of irrigation. At first relatively healthy, such areas are liable to an increasing malarial endemicity that, if it does not altogether nullify the good such schemes bring, at least detracts largely from this' (Christophers, 1924). 'Even irrigation of cultivated fields may be a cause of malaria, and the history of irrigation in Southern California has made it plain that if irrigation works are not to become producers of malaria, drainage must proceed *pari passu* with irrigation; when this is not done, the water which brings riches brings also malaria' (Marchiafava and Bignami, 1900).*

The effects of water-logging in certain areas of the Punjab are graphically described by Gill (1922). Canals at first brought prosperity, but without an efficient drainage system*, water-logging began, 'kallar' (alkaline salts) appeared on the surface of the soil, and 'thereafter the prosperity of the district underwent eclipse until finally the villages became depopulated, malaria increased, wells became useless and the tract, almost wholly water-logged, became, except in a few spots, as bare and barren as the desert'. He notes the

* In some areas in Sind, the absence of an effective drainage system, in connection with the Sukkur Barrage Scheme, appears already to be giving rise to water-logging with a resultant increase in malarial prevalence.

dwindling population, the extremely high spleen rate, and the general signs of misery and decay.

More than half a century ago, Taylor (1870) recorded the pitiable condition of the people in a water-logged area along the Western Jumna Canal.

'The spectacle too of sick women and diseased children crouching among the ruins of their houses (for in many cases the rafters of the houses have been sold), of haggard cultivators wading in swamps, or attempting to pasture their bony cattle on the unwholesome grass is present in his mind constantly'.

The effects of water-logging caused by irrigation, with a resultant high malarial morbidity and mortality, have also been recorded in relation to the Mon Canal in Burma (d'Rose, 1912; Stewart, 1912; Williams, 1912). D'Rose (1912) gives a vivid description of the outbreak :—

'The number of cases gradually increased, and assumed enormous proportions this year, so much so, that 90 per cent of the population have had fever some time or other; with the result that although the crops are good, the people, on account of the declining health and the large numbers of deaths, are unable to reap satisfactorily'. 'In going round the villages one cannot fail to be struck by the number of people one sees with big bellies and spindle shanks, with sallow, dry and unhealthy looking skin, covered with dirt; some of them can hardly walk; others again have swollen feet and legs and a bloated appearance. The village itself is quiet and the people seem to have no life, considering that the Burmese are a happy-go-lucky race. The appearance of the children especially is pitiful'.

The effects of badly designed or uncontrolled irrigation schemes have also been felt in the Madras Presidency :—

'It cannot, however, be too emphatically stated that the need for such irrigation works by no means implies the need to keep them in such an utter state of neglect as to be a constant source of danger to the general well-being. Even as the malaria investigator has got to view with sympathy the requirements of the agriculturist and the labours of the Revenue and the Public Works authorities on his behalf, the latter are equally to realize how necessary it is to ensure that the very facilities that are intended to give the ryot his daily bread do not act as his "death trap" as well. And it cannot be sufficiently brought home that a close collaboration between the two authorities would alone conduce to the optimum well-being of the people' (Rao, 1929a).

The effects of such sickness and agricultural depression are that 'cultivators began to desert their homes in search of livelihood elsewhere. The less adventurous of them, or those whose vested interests kept them back, fell an easy prey to malaria. With their lowered vitality, they failed to recover fully' (Lal and Shah, 1933). Such a desertion of malarious areas is also described by Kenrick (1911) in the Central Provinces, and has been recognised in other parts of the world. 'The rich, the capable and the energetic seek healthier homes, and so the inhabitants of a malarious district tend to become a mere residue of the poor and wretched' (Jones, 1909).

Since Christophers (1925a) wrote his report upon the experimental sugar plantations at Nalbari in Assam (*vide supra*), this very promising venture, which might have led to the exploitation of a very fertile tract of land, has had to be abandoned on account of malaria.

The havoc caused to agriculture in the United Provinces by epidemic malaria has been described by White (1909) as follows :—'the effects of the (1908) epidemic on the agricultural population were deplorable. They could not reap the *kharif* harvest at the proper time, nor could they prepare and sow as large an area of the *rabi* harvest as would otherwise have been possible'. As noted above, d'Rose (1912) also reports this inability to reap the crops during the epidemic on the Mon Canal in Burma.

Malaria 'tends to abound most in the most fertile countries, and at the season most suitable for agriculture' (Ross, 1911). The great fertility of these tracts is a special attraction to settlers wishing to start agricultural operations. This attraction is enhanced by the fact that, in many such localities, the cost or rent of land is relatively low, in proportion to its agricultural potentialities. As pointed out above, many of these very fertile tracts have had to be abandoned, or remain uncultivated or imperfectly cultivated, because of the obstacles which malaria places in the way of settlers and commercial concerns. Practically all the obstructive action caused by this disease is connected with its effects upon labour forces.

(3) SPECIAL LABOUR FACTORS IN RELATION TO AGRICULTURAL DEVELOPMENT.

The disastrous effects of malaria in causing a diminished efficiency and output of labour forces have been discussed in detail earlier in this paper. In addition to these general factors, one has also to consider certain other special ones which have a marked influence on agricultural development. The main ones are (i) the fact that the season of maximum malarial incidence may fall at a time when labour is most needed for planting, cultivating or harvesting crops, and (ii) the fact that a large turnover of labour is a very serious factor in many agricultural undertakings.

(i) *The season of labour inefficiency.*

'This process will be accompanied by great economic loss, for extremely fertile districts, which are the peculiar prey of malaria, may fall altogether out of cultivation. The ruin of agriculture is a great blow to any country, and it must be remembered that malaria attacks farmers in particular, and that mostly at harvest-time, when all their energies are specially needed' (Jones, 1909).

'In the Campagna both man and beast were struck by the disease during the busiest periods of the year for agriculture, namely, in summer and autumn'. 'Men were compelled to flee into the hills in order to save their lives—an expedient which, however, did not always prove successful. A few pale ghosts of men remained, under the beating rays of the sun, to watch over the few wild beasts which resisted the fever according to the law of the survival of the fittest' (Celli, 1933).

The neglect of agricultural operations at certain seasons may be a very serious matter. The inability to plant or tend a crop at the proper time may result in its failure, or a poor yield. Failure to harvest it when ready may result in its damage, and in the failure of the agriculturist to obtain the best price for his products.

The situation has been summarised by Van Dine (1916), who says :—

'The effect of loss of time upon the crops can be measured by the ratio of the time lost through malaria to the difference between the available labor and labor requirements of the crops. It must be conceded that any loss of labor from any cause in the face of surplus labor that exceeds the time lost cannot be considered as operating against the crops. In the case of no surplus labor or an actual deficiency, any time lost through malaria reacts at once on the crops, the seriousness of the neglect of the crops depending upon the period of planting, cultivating and harvesting the crops that the lost time occurs. *It will be shown that time lost through malaria during at least four months of the year falls at a period when there is a deficiency of labor and when the demand of the crops for labor is greatest*'.

Rose (1919), in speaking of the cotton crops in the Southern States of America, points out that, at cultivating and picking times when labour is greatest in demand, malaria is most severe. A delay in picking may result in a failure to catch the early market before the seasonal prices fall.

The shortage of labour caused by greater malarial prevalence during the harvest season has been especially felt by the tea estates of India. Bentley (1908) speaks of 'the leaf too often running away in the midst of the plucking season, or the bushes failing to give their proper outturn for want of cultivation which you cannot always give them'. Macdonald and Chowdhury (1931) draw attention to the fact that 'in tea gardens there is a shortage of labour, few of the gardens being able to secure sufficient for their needs. The annual occurrence of an epidemic at the time of the year when labour is most needed, the plucking season, is a source of direct financial loss to the gardens'. Rice (1931) also points out the part which malarial sickness takes in making a smaller number of coolies, only 65·81 per cent, available in the plucking season, when they are most needed.

It has already been mentioned that White (1909) noted the effects of epidemic malaria in the United Provinces in preventing the agricultural population from reaping the *kharif* crop at the proper time, and from preparing and sowing the usual acreage for the *rabi* crop. Even in the absence of any severe epidemic conditions, the usual seasonal rise in malarial incidence has a similar action, although to a lesser degree.

In many parts of India, the demand for agricultural labour may be great at certain seasons of the year, and the occurrence of excessive sickness at such times may make it difficult to obtain help. Even if workers be available, their efficiency and output of work may be considerably lowered by the effects of malaria. This may result in a higher cost of production and a delay in placing the crops on the market, with a consequent financial loss to the farmer. If labour has to be imported from other areas to meet the deficiency, this increases the cost of production, and these workers may also fall victims to the disease within a short period. All these factors add more to the cost of production and increase the loss.

(ii) *The turnover of agricultural labour.*

As was pointed out by Daniels and Wilkinson (1909), 'there are few diseases which affect the working strength of labour gangs so much as malaria. Attacks of the disease in persons once infected are frequent, debility results, and much sick leave is required, so much so that a double staff of Europeans has to be provided. The mortality is high, though relatively not so high as the morbidity'.

Fuchs (1922) points out that 'there is a large turnover many times during the season by malaria-infected workers leaving mill and farm when they become too sick to work. The situation becomes serious where new labor must be trained in skilled and semi-skilled work'. 'The results are uncertain labor, slower completion of a job, and work of poor quality'. The same subject is stressed by Johnson (1926), in discussing the economic losses caused by malaria—'Do you know that in 1925 the average labor turnover on plantations (in the United States) was 35 to 40 per cent? This is a source of loss on the farm and is the result of discontent. The more prosperous plantations show the lowest turnover and possibly inability to make money with bad health may be at the bottom if sifted thoroughly'.

* This is expressed by Cantlie (1921) in the statement that malaria is to be regarded as the enemy of the employer rather than of the insurance company.

The effects of malaria in causing a large labour turnover have been specially stressed by Watson (1921), in connection with the rubber plantations in Malaya.

This author states 'I believe that the labour problem is nothing but the malaria problem, and that the solution of the malaria problem will also be the solution of the labour problem. No estate can ever have an assured labour force where the women wail, "We cannot have children here, and the children we bring die". Such is the cry on the unhealthy estates: "It is vain to contend with the instinct of her who weeps for her children and will not be comforted"'. 'Unhealthy estates are perfectly well known to the coolies both here and in India, and are generally avoided; not only have these estates difficulty in obtaining labour, but the annual loss of labour through death, discharge, etc., is 30 to 50 per cent greater than on healthy estates'. He gives instances of estates where more than half a newly recruited labour force were unable to work within two months after arrival, while a month later only about one-sixth were working and three-quarters had deserted. 'While the coolies remain on an average three years on the healthiest estates, they remain only two years on the most unhealthy estates. The extra year means, for the healthy estates, an increase of the labour force by 50 per cent, and more than a 50 per cent increase in the work done; for the coolie at the end of his two years is a skilled workman, especially where he is tapping. Malaria is, therefore, an economic factor of great importance'. 'You start in gaily expecting great gain; but somehow fever strikes it (the plantation) in the first year, and before you know where you are, you have spent double the estimate'. Watson also draws attention to the effects of malaria upon the superior staff of rubber estates. Large numbers of these fall victims of the disease, and if they do not die, many of them spend long periods in hospital or on sick leave. 'So much sickness among the Europeans means that they cannot supervise their work properly; and if there is much sickness among the labour force at the same time, the absence of the controlling and directing force of the estate is, indeed, deplorable'. 'The welfare not only of the estate, but of the labour force, depends, therefore, on the well-being of the manager'. He also found the higher the spleen rate the greater the wastage of labour.

Christophers (1925a) mentions the same problem in connection with the Nalbari Sugar Estate in Assam. 'The liability to sickness among the Europeans results in difficulty in the first place in obtaining the services of these and secondly in constant changes in the European staff, necessitating constant retraining and loss of those trained. There is the same difficulty in recruiting and retaining skilled labour. This applies not only to skilled mechanics, but also to servants, dhobies, etc., so that the effects of malaria in one form or another are constantly in evidence and have a demoralising effect on all'.

The financial loss caused by an excessive labour turnover is well recognised in the tea estates of Assam and Bengal, where the cost of recruiting and equipping a new labourer may be Rs. 300 or more. Macdonald and Chowdhury (1931) point out that 'it is the endeavour of every planter to persuade his coolies to settle permanently on his plantation; an unhealthy garden becomes unpopular among the coolies, who leave it; and new labour has to be recruited in their place'. This aspect of the labour problem has also been stressed by Watson (1921) in the case of the rubber plantations in Malaya, and has been specially noted by many other observers in India.

'The importation of large numbers of "unsalted" new coolies, i.e., if they are imported from non-malarial districts, means that a large percentage die, and that the remainder more or less suffer from malaria and its effects until they become acclimatised. Until they become "acclimatised to malaria" these new coolies more or less live on the hospitality of the Tea Estates and hence in my opinion it is much cheaper to look after the established labour force and breed from "immunes" (Dr. Ramsay)' (Strickland *et al.*, 1928). 'The loss of work year in and year out is incalculable not to mention the indirect effects of unhappiness and discontent and longing to get away' (Williams, 1928). 'The total loss to the tea industry from these causes (the check to the natural increase of the labour population caused

by malaria) must be enormous, for one garden-bred coolie is worth many expensive immigrants' (Murphy, 1928). 'Still, importation of labour is so expensive and garden-bred labour is so much more satisfactory in the long run, that it is every manager's ideal to have his own local colony born and bred on his estate' (Malaria Commission, League of Nations, 1930). Stewart (1926), in his note on malaria in the tea estates of the Bengal Duars, says 'the desideratum of every garden is to have a contented domiciled labour force, which will settle on the garden, make their homes there with their families, so that the father, mother and children will be available for work when required'.

The labour turnover may be very large on unhealthy estates. Forsyth (1928) records the malarial attack rates in 7 tea gardens in the Tezpur district of Assam with a total population varying between about 6,600 and 8,600 souls. In 1915 the rate was 250 per cent, in 1916 it was 268 per cent, in 1917 it was 241 per cent, in 1918 it was 155 per cent, in 1919 it was 121 per cent, in 1920 it was 80 per cent, in 1921 it was 63 per cent, and in 1922 it was 81 per cent*. Ramsay (1930*b*) quotes an example where a batch of 75 coolies bolted *en bloc* about a month after their arrival on a tea estate, because a large number of them had developed fever. A batch of 8 skilled workmen (riveters) recruited about the same time had to be sent back after 4 weeks 'as absolute wrecks' from fever. Rice and Savage (1932) point out that in another tea garden the labour turnover is 10 per cent per annum, in other words the entire labour force is replaced by recruitment every 10 years. Covell (1927) also remarks upon the rapid turnover of labour on the tea gardens and coco-nut plantations in the Andaman Islands.

A large turnover of labour tends to generate a vicious cycle, for not only are workers lost, but there arises an increasing difficulty in replacing these men, because of the unenviable reputation which the estate earns for unhealthiness. The amount of local malaria is also increased by the importation of fresh labour, which may have little or no tolerance to the clinical manifestations of the local strains of parasite, and such new recruits are also liable to introduce foreign strains of *Plasmodium*, with which the resident coolies become infected with resultant increased morbidity.

Such a morbidity and turnover amongst the members of a labour force, more especially if these be skilled or semi-skilled, must result in a great disorganisation of work in agricultural undertakings, unless a duplicate staff or excess labour be available. In the former case the cost to the estate must be very markedly increased. In tea estates, this turnover not only means loss of work, but also unremunerative expenditure in connection with housing, medical attention, drugs, etc.

It is impossible to make any precise estimate as to the financial losses which agricultural enterprises sustain as the result of an excessive labour turnover, more especially when there is a shortage of available workmen, but it must be very large.

The different factors which are responsible for the losses, direct and indirect, caused by malaria to agricultural interests in India have been discussed above. These detrimental effects fall not only upon the large agricultural masses of the country—the small farmers and the field labourers—but also upon the larger commercial undertakings. The bulk of the financial loss falls upon the masses, and how great this may be is impossible to estimate, but it must be

* The decreased incidence in the later years followed upon the adoption of strenuous anti-malarial measures.

unbelievably large. Some workers, however, have attempted to calculate the losses to which various commercial agricultural concerns are subjected, on account of the prevalence of malaria.

'The planter loses directly and indirectly. His help is poor; probably he has to stand the expense of sick labor, and certainly his labor is less than 100 per cent efficient. Malaria strikes his plantation just when cotton prices are highest and when labor is hard to get; it curtails his production and he has to gamble with the weather for what cotton he gets later. He gets less for his cotton; his labor gets less; all have less money to spend on the necessities of life; and he finds it very hard to meet his notes on borrowed money' (Johnson, 1926).

The Malaria Commission of the League of Nations (1930), during their tour in India, were impressed with the fact that 'the hyperendemic areas (of malaria) although sparsely inhabited are very often areas where large plantations and large industrial undertakings are situated, and which are therefore often the site of a considerable immigrant population coming from other districts. It is in these hilly districts covered with forest or jungle, with a sparse population, that the immigrants are quickly mown down'. These are the type of areas which are usually most suitable for the exploitation for tea, rubber, coffee, and cinchona, so that the toll of malaria falls heavily upon the labour populations of many such plantations.

The question of the loss on plantations has been studied by several workers in connection with the cotton plantations in the Southern States of America.

Van Dine (1916) investigated the conditions on a plantation of about 1,800 acres cultivated by 74 families. 'Each family cultivated an average of 16 acres. The plantation depended upon the tenants for labor to cultivate (in addition) an average of 8.23 acres each on the day-wage basis. This amounted to a total of 24.23 acres to be cultivated by the labor represented in each tenant family, and equivalent to that of 13.51 acres of cotton. The total loss of time in 13.79 families is equivalent to that of the total crop of 186.3 acres of cotton. With an average yield of one-half of a bale of cotton per acre, this would equal a total loss of 93.15 bales. Allowing \$70 a bale for lint and seed, this would amount to \$6,520.50'. As there was an average of 4 persons per family this is equivalent to about \$120 per person. Rose (1919) states that in one plantation the manager attributed a loss of \$5 *per capita* from doctors' bills to malaria, while the loss to the tenant and the landlord in crop return is much larger. 'As the landlord must look to the tenant's crop both for return on his capital investment and for reimbursement for his large current advances to his tenant families, he is most deeply concerned in any condition that impairs the health and efficiency of the workers on his plantation'. Derivaux, Taylor and Haas (1917) tried the effect of quininisation. They report that the economic loss suffered by the uncontrolled group in their experiment was \$2.52 *per capita*, as compared with \$0.06 in the quininised population. Gray (1919), in an irrigated district in California, states that, of the losses caused by malaria, the labour loss was on the average the most important, medical service was second and medicines third. In a population of 1,081 persons, he calculated the cost at \$27.20 per sick person or \$17.80 per individual of the population.

Ross (1911) states that Dr. Bolton estimated that on the sugar plantations of Mauritius the coolies* lost Rs. 1,25,000 and the planters Rs. 6,25,000 per annum due to malaria. He calculates that the island of Mauritius with a population of 383,000 loses about Rs. 10 lakhs per annum or about Rs. 2.6 per head. At the same rate the population of India would lose about Rs. 9,000 lakhs, or about £69 million sterling per annum.

* The total coolies on the sugar estates is given as about 39,000.

Ross (1926) gives figures showing that, on five rubber estates in Ceylon, where there was a shortage of labour due to malaria and so a resultant failure to tap many of the trees, there was a loss of £1,000 in one month from this cause. These losses varied from *nil* on the healthy estates (spleen rate only 15 per cent) to Rs. 5,579 in the most unhealthy one (spleen rate 76 per cent).

Bentley (1908) states that he estimated that, in a certain group of tea gardens in Assam, there was a largely preventable loss due to malaria of £2 per acre per annum. Rice (1931), in a tea estate in the Bengal Duars, calculated that, with a labour force equivalent to an average of 370,425 working days per annum, the cost of the labour lost was Rs. 60,736, or 8·5 per cent per annum of the capitalised value of the coolie labour force. Rice and Savage (1932), on another estate in Assam, estimate the annual loss to be Rs. 36,201. 'Working on the "Eight Years" Purchase of Profit Valuation basis, the capitalised value of the loss caused by malaria to this estate became Rs. 2,89,608', on an estate the total valuation of whose tea acreage was Rs. 7,14,000, *i.e.*, nearly 40 per cent of the value of the estate without its labour force.

These estimated losses give some indication of the enormous expense which malaria must cause not only to large commercial undertakings in malarious areas, but also to the rural masses of India.

(4) DISCUSSION AND SUMMARY.

Agriculture is the most important industry in India. 'In a country like India no single factor in its prosperity can be so important as the increase of locally grown food supply. Such increase is brought about by improved methods of agriculture, but also by bringing new areas under cultivation. In efforts to open up new areas of cultivation, again disease may often be a factor determining success or the reverse' (Christophers, 1924).

Agriculture loses incalculable sums, because of the action of malaria in causing loss of work, inefficient labour, and the abandonment, imperfect cultivation or retarded development of large fertile tracts. These conditions caused by malaria have led to agricultural decline in many areas, a lower yield of the crops*, and a higher cost of production. These are factors which are preventing India from taking that more prominent place among the nations of the world to which her huge population and natural resources entitle her.

During the 60 years which have elapsed since the first Indian census was taken in 1872, the population of this country has increased by 46·6 per cent. As a result, a problem of the greatest national importance has arisen, as to how these increasing millions are to gain a decent livelihood in the future. A problem of a similar nature, but of less gigantic proportions, has arisen in Italy during comparatively recent years. In the latter country, there were large tracts of land which were very fertile but poorly cultivated and sparsely populated, on account of the prevalence of malaria. Enormous schemes have

* The *Hindustan Times* (Delhi) in its issue of 23rd September, 1934, gives the comparative average yield of rice per acre as 5,700 lbs. in Spain, 2,100 lbs. in Japan, 3,300 lbs. in Italy and only 800 lbs. in India. While this poor yield in India may be due to a great extent to different and less effective methods of agriculture, one is tempted to ask in how far is this backward state of affairs due to lack of enterprise and diminished labour efficiency resulting from the action of malarial sickness upon both the mind and the body?

recently been launched and perfected to rid these tracts of the disease, and so make them available not only as an outlet for the excess population, but also to exploit the natural resources of the country and thereby increase her wealth and prosperity. A similar procedure in India appears to be one, possibly the most important, means of tackling the problem of over-population, and increasing the prosperity of the country. Apart from any humanitarian reasons connected with the great mortality and morbidity which malaria causes in India, it is almost certain that economic pressure will force the malaria problem before the public in the near future, if India is to retain her position among the nations of the world.

(c) THE EFFECTS OF MALARIA UPON INDUSTRIAL DEVELOPMENT.

As remarked by Christophers (1924)—‘Next to the production of food supply in importance to India is the expansion of her industries. Industrial success is largely bound up in the maintenance of effective labour forces, and the factor which determines the satisfactory maintenance of a labour force more than any other is the prevalence of disease. It is the aim of industrial concerns to have a labour force living happily on or near the estate. Very often on account of disease, such communities have to be maintained by constant recruitment. The children die, the adults, except such as weather the storm and become old immune hands, suffer from fever and anæmia and the mental consequences of such a state, and the net result is disease and a deficient and ineffective labour force. In such industries also as employ mill-hands, the influence of disease must be ever present reducing efficiency, increasing cost of wages and limiting profits. We may say that in the matter of industry disease is not only a tax, but to some unknown extent, depending on the industry concerned, a limiting factor to its full and useful development’.

This subject has also been discussed by Le Prince (1932)—‘Let us assume that in one of these progressive countries a group of bankers and prominent business men started to develop a plant to manufacture structural steel or some other important industry. If imported skilled labor should be used, then unless the directors of the industry take adequate precautions, it may not be long before the workmen become infected (with malaria), become inefficient: the operation costs climb out of all proportion to what they should be, skilled labor may have to be temporarily replaced by unskilled labor, accidents will follow and financial difficulty may follow much quicker than is anticipated’.

The report of the Royal Commission on Labour in India (1931) says ‘during our tours we could not fail to be impressed with the tremendous importance of malaria in connection with the health of the industrial worker’. Clark (1934) expresses the opinion that malaria is ‘the disease of greatest economic importance to business operating in the lowlands and river valleys of our (American) tropics’, i.e., in malarious areas.

While industrialisation, in the western sense, has made comparatively little progress in India, yet this country has already felt the harmful influence of malaria on these activities.

The main large manufacturing industries in India are jute and cotton mills, apart from iron and steel production which will be discussed in relationship to mining. The textile factories are mainly situated in large towns, and, as has

been emphasised many times in this article, malaria is mainly a rural disease. Under these conditions, malaria has not affected these industries to the same extent as it has agriculture. If, however, it be proposed to expand the industries and manufactures of India along western lines, this country will certainly encounter the same difficulties as have been met with in this connection in other malarious countries. The industrialisation of many parts of the Southern States of America has been hampered by malaria, and factories located there have frequently been unable to compete with the prices of products manufactured in more healthy areas. The difference between the industrial activity in the healthy parts of Italy, as compared with the malarious ones, has already been mentioned.

In recent years some of the jute mills in the neighbourhood of Calcutta have suffered from a severe malaria epidemic due to the spread of that notorious malaria-carrying mosquito, *A. sundaicus* (*A. ludlowi*). This epidemic has been specially reported upon by Iyengar (1931b) :—

‘During the autumn of 1930, an epidemic of malaria of a severe type occurred in the industrial area south-west of Budge-Budge on the left bank of the river Hooghly. The epidemic was a severe one and affected the entire resident population of three mills, Lothian, Albion and Orient, and the rural areas adjoining them. Practically every one residing in this area was laid up with malaria and a large proportion of the population suffered from several severe attacks of malaria during the season’..... ‘The incidence of sickness among the labour force as a result of this epidemic was so very high that it even affected the outturn of these mills. In one of the mills situated in this area, a considerable fall in the production occurred through invaliding and loss of efficiency among the labour force as a result of this epidemic of malaria’. Iyengar (1931) also reports a high incidence and a severe type of malaria among mill-workers at the Ludlow Jute Mill at Chengail in the Howrah district.

Iyengar (1931a) points out that ‘the situation in the mill area here is very serious indeed’..... ‘If present conditions are allowed to continue a considerable proportion of the workers, both European and Indian, employed in the mills will be largely incapacitated for work, and very soon it may be found extremely difficult to retain much of the Indian labour force on the place’. Bentley (1931) also remarks ‘I cannot too strongly emphasise the importance of checking the spread of this dangerous species of *Anopheles*, as the Port and City of Calcutta and the mills are situated in an area which is potential for the breeding of this species’. The situation was so alarming that in November 1931 a deputation from the Bengal Chamber of Commerce waited upon His Excellency the Governor of Bengal to draw attention to these outbreaks of malaria, and the danger which would arise from their spread. Commercial, railway and shipping concerns were so seriously threatened that a number of these joined together and raised funds to start an anti-malarial campaign in the vicinity of the areas where their interests were situated.

Bombay City has a large number of cotton mills which afford the chief source of employment in that area. Bentley (1911b) has called attention to the losses which occur to the industries of this city on account of the incidence of malaria there. Covell (1928) noted that ‘the correlation between the intensity of malaria and the proximity of mills was most striking’..... ‘In a large part of the Worli and Parel sections, malarial conditions approach those encountered in hyperendemic areas in other parts of India’. This worker calculated in 1928 the average annual cost of malaria in Bombay at not less than Rs. 50 lakhs, and from the importance of the cotton industry to this city, a very large amount of this loss must be borne by it. Christophers (1924) has

also expressed a very strong opinion about the possible effects of malaria upon the commercial activity of Bombay :—

‘As regards the effect on trade a very little experience shows one the effects of disease in limiting and making more expensive all forms of commercial activity. Latterly in Bombay there has been a very ominous increase of malaria in the city itself. Should such conditions increase, as well they may do, in one way or another, almost imperceptibly perhaps, but every day and in every way, to parody a popular phrase, malaria must out in the long run human activity, until in place of a once active and prosperous community there will remain but a pallid remnant dragging out an existence of temporary exile in a decayed tropical metropolis—like the dreams of some Wellsian romance’.

Banerjee (1916) describes the effects of malaria upon the working of a large munition factory at Ichapur. ‘The factory employ 3,000 men a day. Six weeks ago 1,000 men, representing one-third of the total number of workmen, were absent owing to the prevalence of malaria. The Superintendent of another factory near Calcutta said that one-half of his men were absent in the malaria season owing to the prevalence of malaria’..... ‘It seems to me that this is a state of things which deserves the most serious consideration of the Government of India. What a wastage of energy and loss of national efficiency all this represents’.

Many people appear to advocate a policy of industrialisation as a solution of the employment problem in connection with the increasing masses of population in this country. While such a form of development may help to solve the problem, the full benefits of such expansion will almost certainly be hampered in very many localities, by the presence of malaria. The effects of this disease provide the greatest labour problem with which India has to deal. Unless more active measures are taken to cope with this scourge, it will continue to interfere seriously with the ability of the products of this country to compete, in the markets of the world, with those manufactured in more favourably situated countries.

The loss which malaria already causes to the comparatively few large manufacturing industries in India is probably enormous, and forms a serious obstacle to their full and useful development.

(d) THE LOSSES CAUSED BY MALARIA TO THE DEVELOPMENT OF MINING INDUSTRIES.

Malaria has formed a great obstacle to the development of the mineral wealth of this country in many localities. Some of the wealthiest deposits, iron, coal, manganese, copper, gold, mica, etc., are to be found in hilly areas, such as those which run from Chota Nagpur southwards through the Agency Tracts of Madras. As can be seen from the malaria map prepared by Christophers and Sinton (1926), this tract is one of the most malarious in India.

The disease has exercised a baneful effect upon the development of these industries. It is also quite possible that there may be other equally rich mineral deposits in other parts of the hilly and forest tracts of India, which have not yet been discovered, or have remained unexploited, because of the difficulties caused by malaria. Many of these areas have never been properly prospected, because of the intensity of the disease. The difficulties of survey work in such malarious areas have been mentioned by Dr. Otis Smith, the Director of the United States Geological Survey :—

‘In one of the Southern States, 11 topographic parties have been at work during the past field season. The full quota for these parties would be 55 men, but I believe that

something over 100 men have been employed at different times during the season. While I have not the exact figures before me, I feel warranted in the statement that at least 95 per cent of these employees have been sick, for periods ranging from a few days to two weeks, in hospital. Many of them have been able later to return to work, but at least 30 per cent had to leave the field permanently. By reason of this sickness the efficiency of the parties was reduced, at a very conservative estimate, by 25 per cent'. (Howard, 1909).

These areas of the United States are much less malarious than most of the tracts where the mineral deposits are found in India. McNabb and Stewart (1927) took a survey party of United States engineers into a malarious portion of the Isthmus of Panama in the vicinity of the Canal Zone. In spite of military discipline to ensure that precautions should be taken against disease, the infection rate was almost 50 per cent of the men exposed.

The tract of country, through which the Vizianagram-Raipur Railway line was run, is of the same hyperendemic type as that where many of the mineral deposits have been discovered. The disasters, which the parties surveying this line met, will be described later.

Apart from the difficulties which malaria places in the way of prospecting parties, this disease has been found to form a most serious obstacle to the economical development of the deposits which have already been located. The exploitation of these minerals is very important, not only to the private interests concerned, but also to Government and the country as a whole.

Rao (1929a) notes 'among the instances of hill malaria (in the Madras Presidency), which are retarding the steady opening of the country for exploring the vast mineral and natural resources, mention may be made of the manganese-mining area at Sandur'. He also states (Rao, 1929b) that 'extraordinary rich hæmatite and excellent manganese ore occur here and in the neighbouring hills'. 'The work is greatly hampered on account of malarial fevers among the coolies, both resident on the plateau and those recruited from neighbouring villages'.

Very many of these mines are situated in districts which are so sparsely populated that the demand for labour cannot be met locally. This makes it necessary, in almost all cases, for each company to import labour from outside sources. This is especially so in the case of skilled workers, for the local inhabitants are usually very backward, in so far as education of any kind is concerned.

The incidence of malaria among immigrant labour imported into these highly endemic localities is very high and the manifestations of the disease are usually very severe. This gives rise to a labour problem which seriously embarrasses the work of the mines—deaths and excessive sickness lead to inefficient and deficient labour, desertion of workers, difficulty in recruitment, etc., resulting very often in a great disorganisation of the industry, or, in extreme states, to an abandonment of the enterprise as economically impracticable. As pointed out above, the brunt of the disease falls upon the immigrant population, of whom the most important are the more highly paid administrative and skilled technical staff. A high rate of morbidity amongst these will lead to disastrous effects upon the working of the industry.

The damage which malaria causes to the mining industry in the Singhbhum area of Orissa has been reported upon by several workers. Christophers (1925a) and Wats (1924) studied the iron and manganese mines especially. The former observer was asked to report upon the high morbidity among the employees of these mines. He found that this was almost entirely due to

malaria, and the incidence of disease was so high as to interfere seriously with the working of the industry. The sickness and disability was most marked among the superior and skilled staff who had been imported from outside areas. Christophers' investigations were continued by Wats (1924) who confirmed the previous findings and says 'one instance of "incidental malaria" due to the introduction of non-immune labourers into camp was told me by an officer in charge of a mine. About 300 Madras labourers were recruited and sent to work at the mine; in about a fortnight 10 died of malaria and the rest had to leave the area owing to being constantly fever-stricken. Epidemics of this nature make the problems connected with the employment of outside labour in the district difficult, and hinder commercial success generally'..... 'Few diseases affect the working strength of labour gangs so much as malaria, and immigration of non-immune people from healthier districts almost always gives rise to malarial epidemics of some magnitude, hence the necessity of suitable measures'.

In speaking of the iron mines in the same province, Watson (1933) reports, in addition to the morbidity among the mining staff from malaria, an occasion when the furnaces at the smelter were brought almost to a standstill because no less than 7 trains, which had gone for ore, had been abandoned at the mine, through drivers, firemen and guards having gone down with malaria, while the railway staff at the station and the staff at the mine were suffering hardly less severely*.

Malarial sickness interferes with the coal mines in the Burdwan district of Bengal and in the Asansol area of Bihar. The ill-effects of this disease are felt by the labour in the coal mines and oil fields of North-eastern Assam, as also in the lead and other mines on the eastern frontier of Burma.

The Salt Factories in different parts of India provide a very considerable source of revenue to Government. The prevalence of malaria among the superior staff and the labour forces at several of these factories has given rise to much interference with their working. High malarial morbidity, with consequent labour trouble, has been reported in certain of these industrial workings in the Madras Presidency by Matthew (1920), Menon (1925a), Perumal (1923) and Rao (1929c). In the salt fields of Burma, Stewart (1911) has also recorded a high malarial incidence at Sagyin, in the Myaungmya district. The malarial incidence among the personnel at the Government salt works at Khargoda, Kathiawar, was so high that Macdonald (1930) was asked to investigate the conditions.

There is little doubt that malaria exerts a very obstructive action upon the development of the mineral wealth of India. How great the economic and financial losses may be it is impossible to calculate. The baneful effects of this disease retard the progress of the prospector, so leaving much mineral wealth undiscovered, and interfere with the working and development of the mines, so lowering the output and increasing the cost of production. These factors make the mineral products of India less able to compete in the markets of the world; they prevent the development of prosperous industries which would give employment to a large number of workers; and make it impossible

* Watson (1933) also discusses the effect of malaria as a hindrance to the development of the mining industry in other parts of the world.

for the nation to take full advantage of those rich resources with which nature has endowed her. These economic losses must amount to untold millions of pounds, and crores of rupees.

(e) LOSSES DUE TO THE EFFECTS OF MALARIA UPON TRANSPORT AND COMMUNICATIONS.

These effects include not only the direct financial losses to railways, shipping, etc., because of inefficient or deficient labour leading to higher working costs, but also many indirect losses. A rise in the working costs means higher fares and freights, and so an obstacle in the way of the full development of the districts or countries served. In the case of shipping, a high malarial incidence at ports may also lead to restrictions in trading, higher wages for employees, etc.

(1) THE EFFECTS OF MALARIA ON RAILWAYS.

In those countries where malaria prevails, the tax which this disease levies upon railway systems in all stages of their activities has been noted by many observers. Malaria may not only delay for many years the initiation of great schemes of transport, with associated development of natural resources, but may also have a very marked effect in hindering the construction of railways and in increasing the cost of such work. Even after they have been constructed, malaria may add largely to their operation costs, from its effects in reducing the number and the quality of the available labour force. The latter may also require a higher wage and greater privileges on account of its exposure to sickness. This leads to an increase in transport charges, and so an inhibition of the proper development of the natural resources of the area served by the railroad. These factors give rise to a vicious cycle whereby not only is the interest obtained from the capital outlay small, or negligible, but also the high transport charges lead to a diminished traffic, which has little encouragement to increase and so to swell the profits of the line.

The malarious nature of a district may prevent the development of railway facilities in a locality for many years. This may be due not only to the difficulties encountered in surveying the route for such lines, to a realisation of the enormous difficulties which the disease would place in the way of obtaining and retaining an adequate labour force for the construction, and to the great expense which this would entail, but also to the difficulties which the disease would place in the way of the commercial development of the area served and upon which factor the profits of the railway would depend.

Senior-White and Newman (1932) state that 'during the survey of the line at Laing-Biang (Indo-China) in 1900, there was in the eight months a mortality of 77 per cent in the Europeans and 80 per cent in the natives'. The former author (Senior-White, 1928) has described the difficulties and disasters that malaria caused to the parties which attempted to survey the course of a railway line through the very malarious hill tracts of the Madras Presidency from Vizianagram to Raipur in 1883, 1897 and 1907. These parties were unable to complete the survey because of the ravages of malaria. The successful party in 1923 spent only 3 months in the field (January to March) and 'the engineer in charge of the party left railhead with all his staff duplicated. On completion, only 25 per cent were effective'. 'Meanwhile, another survey

across Jeypore State to the west of the line under consideration had cost the lives of two officers of the party'. The difficulties encountered in the early surveys helped to hold back the construction of this line for 40 years.

While malaria may place great obstacles in the path of survey parties, these difficulties only afford some indication of the obstruction which this disease may cause to the construction gangs. The financial loss at the former stage is infinitesimal as compared with that in the latter.

'It will probably be admitted by all in a position to judge that there is nothing so expensive as ill health among the labour forces on the construction of a line; it is impossible to estimate the financial loss to Government of say two or three good construction engineers being invalidated home on account of a preventable disease. In how many instances are estimates exceeded and difficulties multiplied tenfold due to sickness amongst the labour? Therefore, from a business point of view, everything should be done to maintain the whole of the employes, from the Chief Engineer down to the lowest paid coolie in a good and efficient state of health' (Clemesha, 1917).

'Whilst it is a commonplace of the textbooks that railway construction in the tropics, with its "aggregation of labour", is nearly always associated with fulminant epidemics of the disease (malaria), yet in the past little has been done to obviate such happenings'..... "'A death a sleeper" is a vivid generalisation on the happenings' (Senior-White, 1928). As instances of such events in other countries, Senior-White (1928)* quotes the construction of the Beira Railway in East Africa, and the Indo-Ceylon connection of the Ceylon Government Railway. Bostock (1911) notes that the heavy death rate during the building of the Pretoria-Delagoa Bay line forced malaria upon public notice. Thomas (1911) draws attention to difficulties which malaria placed in the way of the building of a line along the Madeira River, to connect Bolivia and Brazil—'Several attempts have been made to build this line, and each time malaria has raged among the workers. A most virulent form of fever occurs, which has at times incapacitated 50 per cent to 80 per cent of the total working force'. Senior-White and Newman (1932)* also cite the instances of the Congo and the Indo-China Railways:—

'In Yunnan, in 1905, in the region of the Lower Namti, in 5,000 coolies from Tien-Tsin who were working on the first 15½ miles, there were about 3,000 casualties, almost all due to malaria. Many died on the spot and gangs abandoned the place, dying along the road while they attempted to flee from this murderous locality. In 1907, in a gang of 170 which had arrived a month and a half earlier, there were 18 dead, 60 ill and 40 bolted. In another gang of 150, 35 were gravely ill, 50 were hardly in a state to stand up and 51 had run away'.

James (1920) reports that, in railway work at a port in Ceylon, the area acquired an evil reputation because of malaria. From the statistics available, it would appear that an ordinary unskilled labourer only put in about 18 working days in the month, and the death rate among the labour force was 46 per mille of the average daily strength.

India has not been without her tragedies and difficulties during railway construction in the past. McCombie Young (1911) remarks upon the very high mortality among the coolies employed in building a branch of the Eastern Bengal State Railway through the Malda district of Bengal in 1903—1907†.

* These authors have described in some detail the relationship of malaria to many phases of railway activity.

† McCombie Young (1911) attributes the epidemic conditions, which appeared among the local population of the Malda district at this time, to the 'tropical aggregation of labour' on the railway works. Clemesha (1917) quotes other instances where railway construction works have been responsible for a heavy malarial incidence in their neighbourhood.

Clemesha (1917), in speaking of the construction of the line between Kishan-gunj and Siliguri in Bengal, states that 'the amount of sickness and desertion on the part of the coolie labour was very high indeed, and the Railway Board are probably better acquainted than I am with the large cost of the work due to delays and enhanced rates to contractors caused by the lack of proper medical arrangements'. Denham (1925) reports a high incidence of malaria during the construction of the Pyinmana-Taungdwingyi Railway in Burma. In one section the number of new cases of this disease in relation to the amount of labour employed was 2.4 per cent in May, but rose to 54.7 per cent in December.

Suhrawardy (1928) points out the necessity for 'reducing the incidence of the disease and the inefficiency caused by it at construction centres', while Senior-White (1928) speaks of the trouble experienced from malaria during the construction of the Ghat section of the Great Indian Peninsula Railway.

The Malaria Commission of the League of Nations (1930) mention 'the Ambda-Jambda line in Singhbhum (Orissa), notorious for the enormous toll of sick and dead from malaria during its construction'.

Senior-White (1928) gives further particulars of this line and says 'the Ambda-Jambda branch of the Bengal-Nagpur Railway, built through the hills of the Singhbhum district of Chota Nagpur in 1923-24, proved yet again what is the price of opening up communications in hyperendemic country without the help of an expert. In the worst length the engineer staff were doubled, in the hope that one officer out of each pair would be in a state to do duty; labour died, or bolted, in such numbers that work was finally undertaken by a Pioneer Regiment from the Frontier. In the circumstances it is hardly to be wondered at that the cost of the branch line considerably exceeded the estimate'. Wats (1924) also gives some details of this outbreak—'from the commencement of the work, the engineer in charge informed me, malaria was rife amongst them (the temporary military labour force) reducing their capacity to 50 per cent and at the end of the work each man had to be given 3 months' leave to recuperate his health'.

Senior-White (1928) reports upon the difficulties which malarial sickness caused in the construction of the Saranda Tunnel, also situated in the hyperendemic Singhbhum area of Orissa. This tunnel was first made in the eighties of last century. No precise information is available as to the malaria rate at that time, but from deduction this must have been very high. 'It is said that mortality and bolting among the labour so disorganised the accounts that the gangs were paid, shift by shift, as they emerged from the workings. Several of the Cornish miners who formed the subordinate tunnelling staff died of malaria, or more probably of blackwater, for which the district is notorious'. As this line serves the iron ore mines of Orissa, it was later found necessary to double the track through the tunnel. Work was started in August 1925, and in this month malaria was responsible for an incapacitation rate of 21 per cent, which rose to 34 per cent in November, when the Assistant Surgeon died of blackwater fever and the office staff was so disorganised that no further records are available until the end of the epidemic in March 1926. By this time all the non-immune labour had practically disappeared, and had been replaced by local aborigines.

These quotations give some indication of the trouble and loss which malaria may cause during the construction of railway lines in areas where malaria prevails. Even when the construction has been completed, the financial and labour difficulties of the company are not finished, for this disease may exact a heavy toll upon the health and efficiency of the staff.

This subject was investigated on the Italian railway systems in the latter part of last century.

North (1896) (quoted by Anderson, 1927) says 'in the year 1879-1880, a Parliamentary Commission was appointed to investigate the condition of the railways of Italy. The Calabrian Railway of more than 500 kilometres in length (310 miles) was found to be almost paralysed by it (malaria), for it not only returned no profits on capital expended, but required large annual subsidies in order to keep it open for traffic. Translated into money, the cost throughout Italy in extra pay, extra labour and medicine, but not including cost of maintenance in hospital, amounted annually to no less a sum than about £600,000 sterling'. Celli (1904) states that according to the very accurate calculations of Ricchi, the Adriatic Railway Company alone, for 6,416 employees on 1,400 kilometres of railway in malarious zones, spends on account of malaria only the large sum of 1,050,000 francs a year.*

Macdonald (1911) says that in one railway company in Spain, 14,275 days' work were lost in consequence of malaria. 'On certain divisions of the line this company keeps double *personnel* to alternate every fifteen days'.

In the United States, Howard (1909) reports that 'throughout the region in question, malaria is practically universal. The railroads suffer, and at the stations throughout the territory it is practically impossible to keep operators at work'. In the same connection, Stanley (1932) says 'the cost of operating many railroads in southern countries is unquestionably higher than need be because of malarial prevalence. This cost, or malaria charge, must be borne by the ultimate consumer of products shipped overseas'.

The epidemic of malaria in the Punjab in 1908 was first brought prominently to public notice by the sudden disorganisation of the train service, due to fever among the employees of the large railway centre, Lahore. At Bombay, 'the guards, porters and sweepers belonging to the G. I. P. Railway were attacked, and so many of the former were prostrated that at one time during the height of the epidemic (1908) as many as ten of the local trains had to be cancelled' (Bentley, 1911b).

Apart from such exceptional conditions, malaria causes a serious obstruction to the efficient and economical working of railway systems in many parts of India. The Lumding Railway Junction in the forest area of Assam was at one time so malarious that its abandonment was seriously considered.

A special investigation of malarial conditions in the railway colony at Myitnge in Burma was undertaken by Williams (1919) on account of the wide prevalence of the disease affecting especially the labour in the railway workshops. Denham (1925) reports that, on one section of the line in Burma, the percentage of railway employees attending hospital monthly for malaria varied from 3 in February to 19.2 in July.

Several medical officers of the Eastern Bengal Railway have drawn attention to the losses which malaria causes to the workers and the administration of this line which serves a very malarious tract of country. Suhrawardy

* The factors in this expenditure are given in greater detail by Senior-White and Newman (1932).

(1928) points out the danger that malaria may break out in a serious form at important and large stations, and 'cause inefficiency amongst important classes of our running and operating staff'. Bishop (1926) studied the effects of malaria upon the staff of the Khulna branch of the same line from September 1924 to March 1925. He reports that, out of a staff of 861 individuals and 1,027 dependents, about 27·3 per cent suffered from primary attacks of the disease, and 11·0 per cent with recurrent attacks. In 18 stations with a staff less than 15 persons each, 50 per cent of this population suffered from malaria during the period under review. In 6 stations with a staff of 15 to 30 persons, the percentage was 40; in 3 stations with a staff of 30 to 100, it was 23 per cent, and in 2 with a staff over 100 it was 16 per cent*. Sladen (1927) records that, at Ishurdi station during the month of September 1925, there were 3,207 days of certified sickness from all causes among a staff of 617 individuals, and, of these lost days, 2,565, or more than 4 per person, were due to malarial sickness. One year later, after anti-malarial measures had been in operation, the total loss was only 696 days during the same month, of which 53 were due to malaria. Sladen estimates that the cost of a man being off-duty from sickness is Re. 1 per diem (this he thinks an underestimate). Taking this figure it means that the Eastern Bengal Railway lost, at Ishurdi alone, Rs. 2,565 during the one month of September 1925. This officer also points out the loss to the railway of sick leave to its staff—'These long absences from duty were and still are a heavy cost to the Administration'.

The subject of the losses due to malaria was also investigated by Rao (1928) in the Lalmanirhat district of the same railway. He estimates that the loss was Rs. 27,367 in this one district during 1924, from days lost due to malarial sickness among the staff, in 1925 it was Rs. 35,957, and in 1926 it was Rs. 24,248, allowing the financial loss to be Re. 1 per diem. The Agent of this line states that, during 1923, out of 48,000 employees, the number of sick treated as outpatients by the railway medical staff was 89,904, of which about one-third were due to malaria (Sladen, 1927). During 1925 there were more than 120,000 cases of sickness amongst the staff and their dependents. 'The railway lost the service of each member of the staff for one week, or, in the aggregate, nine hundred work years, as a consequence'.

Senior-White (1928) gives details of the difficulties which malaria caused at Dangaoposi station on the Bengal-Nagpur Railway—'Things began to go wrong in August, when the line had been open five months, with the onset of the monsoon. Within a few weeks conditions had become so bad that the station was almost at a standstill'..... 'At the end of October there was hardly a single member of the staff who had escaped malaria, which was still raging with almost undiminished intensity. Relief men were doing most of the work and going down themselves'. He gives figures showing the days lost, the percentage of the staff attacked, etc., which 'indicate how complete was the disorganisation owing to absences'. He points out that such outbreaks are characterised by an inordinate delay in getting the traffic moving again when they affect important centres. He also records that 'within the last three years at least three of the largest railways in this country have experienced

* The cost of anti-malarial operations *per capita* of the population is greater at small than at large stations. As pointed out by Senior-White (1928), in such small stations the absence of a man for duty is more felt, and occasions greater disorganisation—maybe in main line train-working—than at larger stations, whilst relief is less easily obtainable.

similar outbreaks, all of them on a large scale, affecting more important division points than the one here described'.

As pointed out previously, malarial sickness is not only of importance because of its action upon the physical condition of the sufferer, but also upon his mental one (*vide* Rao, 1928). The latter aspect of the case has a special influence in connection with railway work, where many of the employees are employed on skilled or semi-skilled labour. Excessive sickness among such personnel may mean that their duties have to be taken over by less highly trained workers, with not only a financial loss to the company, but even a considerable degree of risk in some cases (*vide* Rao, 1928).

This excessive malarial sickness among the staff of certain Indian railways gives rise to an enormous amount of financial loss to the companies concerned. The company has to bear the expenses of sickness among its workers, reduplication of staff, inefficient labour, higher pay and special allowances to workers in malarious localities, extra leave, sick pay, etc., etc. Apart from these direct losses, the company has also to bear those which arise from that hindrance to the development of the natural resources of the area served, which is caused by the presence of an expensive, and sometimes defective, system of transportation.

It is apparent, therefore, that malaria exacts a heavy financial toll from many of the railways of India. This disease, both directly and indirectly, hampers the company from the period of the preliminary survey, through its construction, and, even after the line is completed, adds much to the running costs. All these factors lead to incalculable financial losses not only to the shareholders* of the railway, but also to the country at large. The latter aspect of the problem is largely connected with obstruction to the development of the natural resources of malarious areas.

(2) EFFECTS OF MALARIA ON SEA-BORNE COMMERCE AND SHIPPING.

The harmful effects of malaria on shipping, etc., may be manifest, as in the case of railways, in the expenditure needed to construct harbours, docks, ship canals, etc., in the upkeep and working costs of these after completion, and in inhibition of trade and commercial development.

An Editorial in the *Journal of Tropical Medicine and Hygiene* (1910) draws attention to the sudden exacerbation of malaria which occurred at Hong-Kong during the works which converted the site from an obscure Chinese village into an important modern port.

When Port Swettenham in Malaya was being built, there was a considerable amount of malaria among the workmen (Watson, 1921), and this must have interfered with the work. The construction of the Ocean Harbour at Belawan in Sumatra was started in 1917, and in 1919 a fulminant and very severe outbreak of malaria occurred among the workmen. Schuffner and Hylkema (1922) have estimated that more than 10 per cent of the people died from June to September.

The French attempted to build the Panama Canal, but were driven out by malaria, yellow fever and dysentery. The labour difficulties caused by sickness

*In India the Government is the chief shareholder in the railway companies, so the effects of malaria must have a very serious action in diminishing the revenue from this source.

were so great, and apparently insurmountable, that the company concluded the canal was not worth the sacrifice and were glad to sell their rights to the United States as a bargain. The latter country, by scientific anti-malarial measures, were able to complete the task, and make it pay.

The Alexandra Dock and Hughes Dry Dock at Bombay were commenced in 1904. The malarial incidence in the vicinity was noticed to be increased in 1907 (Liston, 1908). Bentley (1911b) reports that 'in 1908 the disease broke out with increasing severity and as a result there was an exodus of coolies and labourers employed in the neighbourhood of the docks and among the shipping'. 'The headman of one gang volunteered the statement that of 300 working coolies who came with him from their district (Marwar) one hundred died and another hundred had deserted their work during the preceding eighteen months'.

Even when the work of construction of facilities for sea-borne traffic has been completed in a malarious country, the labour difficulties are not finished and may still give rise to great loss and trouble.

An outstanding example of the effects of malaria upon the working of, and the traffic in, a port is that of Port Swettenham in Malaya. Watson (1921) gives the following account of the epidemic which occurred immediately after the port was opened, and which threatened to cause its closure :—

'Immediately after the port was opened the malaria assumed an epidemic character. In less than a month the 180 leading coolies were so decimated by disease that the remnant refused to live any longer at the port, and returned to Klang'. 'Two months after the port was opened I visited the native houses which had been built; of the 127 inhabitants 78 were said to have been attacked and 25 out of 27 houses were infected'. 'The Government population also suffered severely; out of 176 persons, including the crews of Government yachts and launches, no fewer than 118 were attacked between 10th September and 31st December'. 'The effect of the disease on the business of the port was very serious. Ships came in and could not be unloaded. Those on fixed runs had to overcarry cargo. The crews contracted malaria, and after a month or so it was impossible to obtain a crew willing to trade to the port. The Harbour and Railway Departments were so crippled that they could only imperfectly do their duties, and so utterly demoralised did the port become that the High Commissioner ordered the closure of the port until it could be made sanitary'.

Schilling (1911) reports that in Dula, the most important seaport in the Kameruns, the malarial morbidity amounted to about 391 per cent in 1896-97. In certain months as many as 70 per cent of the Europeans became infected.

Ross (H. C.) (1911) gives the history of the port of Ismailia on the Suez Canal. This port was originally intended by de Lesseps to be one of the greatest ports in the world. The introduction of the fresh-water canal into this area caused a very high incidence of malaria. This was so great that the port was abandoned as a great shipping centre.

'In the days of its salubrity, Ismailia had progressed very rapidly, rising to a population of 10,000; but when the fever appeared, all progress stopped and decadence began. Nearly 2,000 cases of malaria were treated every year, but probably many more occurred. Men, both Europeans and natives, were unable to work, children were always ill, the death rate increased, while the birth rate fell. Every one was down with fever, and trade was soon at a standstill. The Government offices were closed and were ultimately moved to Port Said'. 'and the Suez Canal Company were, in 1900, left to face the question whether the town should be abandoned, or whether the disease could be prevented'. 'Ismailia affords a striking example of the great commercial importance of malaria prevention'. 'De Lesseps' great commercial proposal was disposed of by malaria. For years the Company tried to force ships to coal and land passengers at the fever-stricken

town, but now they have been compelled to relinquish the natural harbour, and to build an artificial one at Port Said at an outlay of many millions' (Ross, H. C., 1911).

A high incidence of malaria in the neighbourhood of large harbours may lead to a diversion of traffic from such ports, not only because of sickness contracted by the crews of vessels, but also because of illness and inefficiency among the shipping agents, the dock labourers, etc., which will make it difficult to obtain labour to discharge or load cargo, or only at a high rate of pay. Such difficulties will interfere with the development and expansion of the port, and of other industries whose activities are intimately associated with it. A port has to pay for a bad sanitary reputation. It may be mulcted by health restrictions, sanitary regulations, etc., which may entail delay in commerce, and perhaps may even direct trade elsewhere.

In Current Topics in the *Indian Medical Gazette* (1900) are recorded complaints from the Port Commissioners of Calcutta and from shipping agents that the work of coaling at the docks was seriously interfered with by an epidemic of malaria among the coolies. Covell (1932) draws attention to the danger which the spread of *A. sundaicus* towards the Kidderpore Docks of Calcutta formed. He reports that shipping and other firms regarded the danger of malaria as of such real importance that they formed an anti-malarial association to combat the threatened invasion. As mentioned previously, Bentley (1911b) records the exodus of labourers from the dock and shipping areas of Bombay, because of the severe outbreak of malaria there.

Outbreaks among the crews of ships using harbours in India have been reported on a number of occasions. Rogers (1906) draws attention to the number of sailors, who had not previously suffered from malaria, but who contracted the disease within a few weeks of coming to the Kidderpore Docks at Calcutta. Bentley (1911b) records that, during the epidemic of malaria at Bombay in 1908, the crews of many of the P. and O. vessels moored in the most southerly berths of the Victoria Docks contracted malaria. On boat from 80 to 100 cases of the disease occurred among the crew during the homeward voyage, while the passengers escaped. The same epidemic is mentioned in an Editorial in the *Journal of Tropical Medicine and Hygiene* (1910).

In former years the port of Bombay was healthy. 'Since, however, the new docks, which have been commenced about five years ago, have been under construction, the docks and their neighbourhood have become progressively malarious, so that the fortnight's stay of the P. and O. liners in dock is now almost inevitably followed by a number of cases in the *personnel* during the voyage home, and the sick rate has occasionally reached so high a figure as to render these splendidly found steamships practically short-handed'.

Another outbreak occurred in the autumn of 1922 among the crews of ships in the Alexandra Dock, which was completed in 1914. Covell (1928) records that similar outbreaks occurred among the crews of ships using this port in 1923 and 1924 also.

From the data given above, it is easy to see how shipping and commerce may be hampered by malaria. A heavy initial expenditure may raise the rate of dock and port dues, labour may be inefficient or deficient, so increasing the cost of working, vessels will tend to avoid such unhealthy ports, or will only accept cargoes at freights higher than those of more healthy ports. All these factors will tend to increase the sale price of materials shipped through such ports, and so will interfere seriously with the development and prosperity of the areas which these ports serve.

(3) SUMMARY.

It is evident, from the information which has been collected in the preceding sections, that the prevalence of malaria interferes very seriously with the staff and labour forces of railway and shipping interests in areas where this disease occurs. As a result a very heavy economic and financial toll is levied by its effects.

By its action on labour (i) it raises the cost of construction of their engineering and other undertakings, and (ii), when these are completed, it increases the operating costs through its tendency to produce a deficient and inefficient staff. These factors lead to (iii) a diminished profit upon the capital invested, and (iv) form serious obstacles to full commercial and economic development of the localities and countries served by these forms of communication and transport.

It is impossible to calculate the direct financial losses which the disease causes to the companies concerned, but they must be enormous. Even greater than these must be the economic losses to the country from the action of this disease in limiting the full development of the natural resources of the areas served.

(f) MISCELLANEOUS.

The effects of malaria upon railways, etc., are but spectacular examples of what malaria is doing continuously, and on an enormous scale, to hinder industrial enterprises. This action of the disease upon the labour problem is felt not only by those undertakings specially considered above, but by almost all employers of large labour forces in the tropics. It is more especially felt where conditions of 'tropical aggregation of labour' exist, and proper anti-malarial precautions have not been taken.

Bentley (1911b) speaks of a severe epidemic at Bombay when the Colaba Causeway was being constructed from 1838 to 1841. Other outbreaks occurred during the Back-Bay Reclamation Scheme of 1861—1866, and also when various water-works were being made in the neighbourhood of Bombay.

Labour forces employed on the construction in the foot-hills of head-works for large irrigation schemes have also suffered severely from malaria. At the Sarda Canal Head-works in the notoriously malarious Terai of the United Provinces, Clyde (1931) says that 'in the first year of the work, before anti-malarial measures were started, work had to be closed down in April because 96 men out of every 100 imported were down with fever at one time. Contractors refused to carry on the work and cleared out one after another, and it was realised that unless active measures were taken the head-works would never be completed'. Among a skilled labour gang imported from Bombay, the loss in man-hours each month from October 1927 to June 1928 averaged 10.8 per cent and was even as high as 23.4 per cent in May of the latter year. Phillips (1929) states that the anti-malarial measures taken prolonged the working season from about 3 months to nearly 8 months each year. Sir Bernard Darley, the Chief Engineer, told the Legislative Council that it was very doubtful whether the head-works could have been built without such measures. He says that by these measures about a year was saved, which resulted in a saving of at least Rs. 50,000 on the pay of staff alone, while on contracts for

masonry, etc., the 'saving may be fairly estimated at Rs. 1,50,000, even assuming that any contractors would be willing to take on the job'.

In the early days of the construction of the Sennar Dam in the Sudan, before anti-malarial measures were started, 33 per cent of the labour was attacked and the death rate was 31 per mille. The labour was largely incapacitated (Atkey, 1926).

These instances give some indication of the loss which malaria may cause in connection with large engineering works in areas where the disease occurs.

(g) SUMMARY AND CONCLUSIONS.

In very many parts of India the malaria problem and the labour problem are practically identical. This disease, by its action upon almost every aspect of human activity, is responsible for enormous financial and economic losses to the individual, to the community, to private enterprises and to Government.

As agriculture is the most important industry in India, and because malaria is pre-eminently a rural disease, it is this branch of human activity which has to bear the greatest burden of financial and economic loss from the action of malaria upon labour. The agriculturist in India, whether working privately or commercially, loses incalculable sums because of the action of malaria in causing loss of work, inefficient labour, and the abandonment of imperfect cultivation or retarded development of large fertile tracts of India. There is much evidence to suggest that, in many parts of India, malaria plays a most important part in producing the low economic status of the rural population, and that, until this burden is removed or largely ameliorated, full and successful development of the agricultural resources of such areas cannot be hoped for.

The labour problems caused by malarial sickness are also felt by other industries engaged in the manufacture of different products. Special details have also been given of the action of this disease upon railways, sea-borne commerce, etc. The profits, which accrue under more normal circumstances from these commercial undertakings, are very largely limited and decreased by the burden which malaria places upon their development and operating costs.

There is no doubt that malaria is responsible for the production of the greatest labour problem with which India has to contend, and that the losses which it produces, both directly and indirectly, must run into millions of pounds annually.

(V) FINANCIAL AND ECONOMIC LOSSES TO PUBLIC ADMINISTRATIONS AND TO GOVERNMENT.

'Apart from the mortality, the disease (malaria) probably levies a heavier tribute on the capacity of the officers and officials of the British Empire than does any other single agency' (Shipley, 1908).

'Not inconsiderable must be the loss to India of increased salaries paid because the world's market fixes its own rates for life endangered by disease, increased expenditure for invaliding, shortened service and increased leave, that the world also demands as its price for exposure to disease. Worse still must be the money paid for ineffective service of subordinates liable to frequent sickness or definitely on the sick list. Here may be added also the cost of maintaining the sick, of supplying drugs, of the upkeep of hospitals . . . and so on' (Christophers, 1924).

The previous portions of this consideration of the financial and economic losses caused by malaria have dealt mainly with these in special relation to the individual, to the community and to private enterprises. When one considers that malaria is the commonest cause of ill health in India, the extra expenses which this disease lays upon administrations and Governments, through its harmful action upon both the physical and mental capacity of their employees, must be very heavy.

Such administrations lose directly through a diminished return from taxation, and also from those enterprises which they finance, *e.g.*, railways, canals, etc. Indirectly there is a great wastage from the higher cost of administration caused by the occurrence of inefficient and deficient service, higher rates of pay and larger allowances, higher pensions granted after shorter service, cost of sickness among the official and general population, etc., etc. Even greater must be the indirect loss to the country from the obstruction which malaria places in the way of the full and proper development of the natural resources of the country, and so of its progress towards increased prosperity and wealth.

(a) LOSS OF REVENUE.

Public administrations and Governments lose revenue both directly and indirectly. The direct losses are mainly connected with (1) diminished returns from taxation and (2) diminished profits from those undertakings which have been financed by Government money. Indirectly they lose from (3) the obstruction placed by malaria in the way of that full and successful exploitation of the natural resources of the country which would lead to the development of large industries, with an increased prosperity of the people, and so of an ability to provide larger revenue to the state.

(1) LOSS DUE TO DIMINISHED RETURN FROM TAXATION.

The rural population of India has been aptly described by Christophers (1924) as being composed of 'communities whose combined resources are woefully small—so poor that they practically cannot be taxed in the western sense'. This absence of prosperity is probably produced very largely by the effects of disease, mainly malaria, upon both the physical and mental progress of the people. Physically they are incapable of undertaking and performing the amount of work which a healthy population can, and mentally they have not the initiative to start such enterprises as would lead to increased wealth through the successful development of the abundant natural resources of India.

While it is impossible to make an estimate of the enormous losses to revenue which are secondary to the physical and mental disability caused by malaria, some idea can be formed of the losses to revenue dependent upon the enormous mortality, direct and indirect, which this disease produces.

From an examination of the total amount of the revenues collected by the Central and Provincial Governments in British India during the financial year 1933-34, it was found that this was equivalent to about Rs. 8 *per capita* of the population. With a *direct* death rate of 1,000,000 persons per annum from malaria, this means that India loses a potential source of revenue equivalent

to Rs. 80 lakhs each year, which is equal to the interest at 5 per cent on a capital sum of £12 million sterling. If to this mortality be added an equal number of deaths indirectly due to this disease, the above estimates are doubled.

Land revenue forms the major item of income in the budgets of the Provincial Governments of India, and, as pointed out above, agricultural interests are those upon which the adverse effects of malaria in India fall most heavily. It is evident, therefore, that the income available from land is much less than that which could be obtained under more healthy conditions. In some instances, when the incidence of malaria is severe, Government has had to remit a large amount of the taxation available from this source. Phillips (1925) has pointed out the case of the Terai of the United Provinces, where the revenue has fallen considerably as a result of the ravages of this disease.

From a consideration of the evidence available, there is no doubt that malaria has a marked effect in limiting the revenue which Government should obtain from the population of India, if this disease were less prevalent.

(2) LOSSES IN UNDERTAKINGS FINANCED BY GOVERNMENTS.

Two of those major undertakings in India, which are largely financed by Government funds, are railways and irrigation. As has been discussed previously*, it is clear that the income derived from railways is seriously diminished by the effects of malaria upon the staff and employees of such systems. Not only is the capital outlay required during construction increased, but the return from the working of the completed line is reduced and its increased commercial prosperity hampered. It is impossible to make any estimate of the enormous loss which railways in India suffer from the direct and indirect effects of this disease.

It has also been pointed out above† that malaria has, in many instances, led to a decline in the prosperity of irrigated areas in different parts of India. This not only leads to a reduced revenue from such areas, but also in much capital expenditure in attempts to remedy the injurious conditions.

‘In the great Canal Colonies, a serious menace is the malaria that is normally induced as the result of irrigation. At first relatively healthy, such areas are liable to an increasing malarial endemicity, that, if it does not altogether nullify the good such schemes bring, at least detracts largely from this’ (Christophers, 1924).

The effects of malaria upon the salt industry, which is also a Government undertaking, have also been commented upon.

The malaria which, if not controlled, plays such serious havoc with ‘tropical aggregations of labour’ connected with the construction of all large engineering works in malarious parts of India, is responsible for a very heavy increase in the capital expenditure needed for the successful termination of such undertakings.

These instances show some of the ways in which capital invested by Government in large undertakings fails to give that return which would be obtained in the absence of malaria.

* *Vide* pp. 471-476.

† *Vide* pp. 456-460.

(3) LOSSES DUE TO HINDRANCE TO THE FULL AND SUCCESSFUL DEVELOPMENT OF NATURAL RESOURCES OF THE COUNTRY.

There is little doubt that malaria is the most important disease which afflicts the inhabitants of India. Apart from the suffering and mortality which it causes, it has been shown to have a most harmful action in hindering the physical efficiency and the mental activity of the inhabitants of those areas where it is prevalent. These effects must exercise a tremendous obstacle to the full and successful development of those natural resources with which India is so richly endowed, and to the development of those industries for which her huge population would fit her.

There is much evidence which suggests very strongly that malaria is one of the chief, if not the most important of the reasons why India has remained a relatively poor country in the past. There seems little prospect that she will achieve to the full that destiny which her huge resources and population appear to indicate as possible, until this appalling burden has been largely ameliorated or removed.

Malaria hinders not only the successful development of the agricultural, mineral and other resources of this country from its action on labour, but, from its effects upon the mentality of the individual and the community, also removes or diminishes the stimulus needed for the initiation of such development.

Even when such schemes have been started, the higher cost of production, caused by inefficient or deficient labour and by diminished business acumen of the producers, seriously hampers the chances of these products in their competition in the open markets of the world. In addition, the sale prices are often raised by the increased cost of transportation to a suitable market, which arises from the effects of malaria upon railway and shipping activities. The trade of a malarious country may also have to pay for the bad sanitary reputation of its ports, etc., and, as is the case with some other diseases, be 'muled by health restrictions, sanitary regulations, and quarantine applied to its shipping and ports, all entailing delay in commerce and perhaps directing trade elsewhere' (Christophers, 1924).

'A recent bulletin gives the value of India's annual imports as over 200 crores of rupees and that of her exports as about 300 crores. A very small *ad valorem* percentage as a result of such influences as we have indicated would very soon run into crores. This is a modest statement of the case against disease in India. The cost I cannot pretend to put into figures' (Christophers, 1924).

All the factors mentioned hamper the full development of trade, the production of wealth and the prosperity of the nation, upon all of which conditions the revenue of administrations and Governments depends. The whole forms a vast vicious cycle whereby malaria hampers progress, and absence of progress leads to an increase of malaria.

The potential wealth and revenue which is lost because of this obstruction to the economic development and prosperity is probably the greatest financial burden which the people of India have to bear.

(b) INCREASED COST OF ADMINISTRATION.

The opinion expressed by Christophers (1924), on the action of disease in increasing the cost of administration in India, has already been quoted at the beginning of this section. As malaria is the most important disabling disease

in India, as in the rest of the tropics, these remarks may be applied verbatim to the malaria problem of this country.

King, in his memorandum of 1911, calls attention to the morbidity and consequent disability caused by malaria in India. He says that this 'aspect of the subject is of special moment in reference to the swelling of pension charges, on account of military and civil officials, who have, in age and service, broken down prematurely'.

The effects of malaria upon the cost of administration have been equally felt in many other tropical countries.

Lugard, in his book 'Dual Mandate in British Tropical Africa' (*vide* Annual Report of the Ross Institute for 1931, p. 38), points out that 'the diseases of tropical Africa are comparatively few; blackwater, malaria, dysentery and anæmia are the principal ones. In West Africa to supply the vacancies caused by the absence of officers on leave in England, one-third additional staff has hitherto normally been required. But if to this be added the deficiencies due to invalidings, extensions of leave for ill health, and local absence from duty for the same cause, the proportion actually available for duty is, or certainly was, probably not more than half. In other words, the revenues must provide double the British staff required for the efficient conduct of the administration'. A correspondent in the *Journal of the American Medical Association* (1930), in speaking of the economic losses caused by malaria in South Africa, says 'in both the northern provinces, it is still customary to induce officials to work in malaria-stricken districts by offering them a "malaria allowance" which is of the nature of a special solatium'.

This increased cost of administration is produced by the action of malaria upon all Government servants employed in areas where the disease prevails, be they connected with either civil or military activities.

(1) HIGHER RATES OF PAY, ETC., FOR OFFICIALS.

As has been noted above, officials and others who are engaged for work in malarious localities or countries, need to be attracted by higher rates of pay, if the services of the best men are to be obtained. In addition to this, often higher rates of pension, earned after shorter periods of service than in the case of healthy areas, are usually offered. Even when the pay and pensions are markedly higher, officials may require to be given special allowances for service in notoriously unhealthy places, for example in the Agency Tracts of the Madras Presidency, at certain railway stations, etc. Service in such unhealthy areas frequently necessitates the separation of officials from their families with much additional expense for which compensation is needed.

Persons stationed in malarious areas usually require to be given specially favourable terms of leave to enable them to recuperate their health periodically, and, in addition to this, the necessity for sick leave and absence from duty due to illness is much greater than in more healthy localities.

The result of these factors is that the service obtained by administrations and Governments may be defective because of the diminished mental ability of the malaria-infected officials, the diminished physical ability of all officials while ill, and the periods of service lost during sickness. All these mean that, for the money expended, the return in service received is markedly less than in a more healthy area.

The cost of efficient administration in a malarious locality is, therefore, a marked tax upon the finances of the administration which employs officials in such places.

(2) EXPENDITURE ON MEDICAL AID, ETC.

The total medical budgets of the nine major provinces of India during the financial year 1931-32 (as given in the *Indian Year Book*) were about 400 lakhs of rupees, or 3 million pounds sterling. While about 16 per cent of the attendances at official hospitals and dispensaries are labelled as 'malaria', it is probable that at least 25 per cent of these are due to this disease. If the medical expenditure on malaria cases is proportionate to that spent on other diseases, at the lower figure of 16 per cent this means that, in such official institutions, Government pays a sum of Rs. 64 lakhs, or nearly half a million pounds sterling, per annum in the treatment of malaria. If the more probable figure of 25 per cent be taken, the sum rises to Rs. 100 lakhs per annum. This sum takes no account of the money spent for the same purpose by private individuals and undertakings. As only about one-tenth of the malarious sick in India attend official hospitals and dispensaries, the expenditure on medical aid by the rest of the population must be several times these sums.

The Annual Report of the Cinchona Plantations and Factories in Bengal for 1930-31 states that 'the total consumption of quinine in India kept remarkably steady at about 211,000 lb. per annum'. If this be valued at the market rate of Rs. 18 per lb., the amount expended by the Government and the public on this one drug must be about Rs. 38 lakhs.

(3) ADDITIONAL EXPENDITURE IN RELATION TO SICKNESS AMONG THE OFFICIAL POPULATION, WITH SPECIAL REFERENCE TO THE ARMY.

While the cost of medical aid to much of the official population is included in the expenditure mentioned above, certain other official bodies, such as the Army, the Railways, etc., keep separate medical staffs, and the cost of these and other medical expenses is payable separately from the budgets of these branches of Government. This means that the expenditure mentioned above is not the only sum which is payable by Government on account of the treatment of malarious sick.

It has not been found possible to make any estimate of the extra expenditure borne by Government on account of medical aid to its civilian officials, but several workers have given some estimates in relation to Army matters.

Mackenzie (1929a, 1929b) gives the 'cost of maintenance of British military hospitals in 1926-27 (including supplies and services)' as about Rs. 114 lakhs. He calculates that 'a sum of over 57 lakhs of rupees (£400,000) is expended *annually* on maintaining in hospitals in India the excess of (British) sick as compared with the number of sick at Home'. This author (Mackenzie, 1929b) states that malaria accounts for about 30 per cent of the admissions to hospital among troops in India, it is therefore probable that this disease levies a tax of about Rs. 34 lakhs annually upon the Army budget on account of sickness among British troops alone. This does not include the cost of medical aid to the even larger force of Indian troops included in the Army in India. If these be taken into consideration the cost would be at least doubled.

As in the case of other communities whose work is largely physical, the labour inefficiency caused by malaria among troops in India must be very high. As remarked by Hoffman (1928) 'the physical efficiency of an army is measured or determined by the constantly non-effective rate, that is the proportion of

men constantly on the sick list'. Mackenzie (1929a) gives the number of British troops constantly sick in hospital as 31·7 per mille, and constantly sick in barracks as 21·86 per mille, while among Indian troops the figures are 18·05 and 15·30 respectively. This means that an average of about 4 per cent of the troops in India are constantly sick. If we take Mackenzie's figure that 30 per cent of the admissions to hospital are due to malaria, this means that more than 1 per cent of the Indian Army is constantly sick, in spite of the precautions which are taken to protect the troops from malaria.

Boyd (1929) points out that in 1923 there were reported, among British and Indian troops in India, 33,521 cases of malaria. 'Suppose that their stay in hospital is seven days, followed by three days' light duty, which is not excessive for an average case of malaria, one finds that 335,210 working days, or over 918 years, were lost in 1923 to the Army alone'. This estimate takes no account of the diminished efficiency which occurs for some time before and after an actual attack of malaria of sufficient severity to send the soldier for treatment.

This loss of efficiency means that a larger force must be maintained to perform the duties which could be carried out by a smaller number of healthy men. This need for a larger number of men is specially marked in the more malarious stations of India and at certain seasons of the year, when the percentage of daily sick from this disease rises to many times the average figures given above.

This wastage of troops is that which occurs under the ordinary conditions of garrison service in India, but one must consider what effect malaria has upon troops performing the duties for which they are specially trained, namely war.

Even during manœuvres, the higher rate of sickness and disability among malaria-infected troops, as compared with more healthy ones, is well recognised. Still more serious are the effects of field service conditions upon such troops. Not only does the great stress of such more strenuous conditions tend to produce acute relapses among infected men who would remain relatively fit under ordinary peace conditions, but these men are unable to perform as much duty in an efficient manner as can healthy men and, as a result, they break down under the strain. Apart from the loss in numbers to the available striking force, the services of many other men are occupied in ministering to these unfits and transporting them to hospital. This may place a very serious strain upon the working of the lines of communication, and upon the operations of the force. In the field a unit which is seriously afflicted with malaria is often more of a drag than a help to the fighting force. The question must seriously be considered as to whether, under certain conditions and in certain localities, it would not be cheaper in the long run to spend money to ensure a small but healthy and efficient force, in contradistinction to a larger but unfit one.

Apart from the expense and disadvantage of employing a force infected with malaria upon field operations, must be considered the question of the expense involved in taking a relatively healthy force to carry on operations in a malarious country or locality.

Examples of cases where the success of military expeditions has been prevented by the ravages of this disease, rather than by the attacks of the enemy, are innumerable and have been recorded from very early times.

Genovese (quoted by Balfour, 1924) is inclined to think that the 'Angel of Death' which 'spread his wings on the blast' and destroyed the Assyrian host attacking the Israelites, may have been malaria. He also states that many of the Carthaginian armies faded 'like mists before the wind' because of this disease, and that the losses among the Roman armies in their wars with Hannibal must have been incalculable. Balfour (1924) notes that Tacitus says that the Gallic and Germanic troops suffered severely when camping in the neighbourhood of the Vatican, and in 1167 the finest army ever commanded by Frederick Barbarossa was decimated in the Roman Campagna by this disease. 'Obscure soldiers, nobles and illustrious prelates laid down their lives there. The flower of a nation was exterminated'. Macdonald (1911), in speaking of malaria in Spain, records that in September 1282, when Philip the Bold of France besieged and took Gerona, his army was almost destroyed by pernicious fever, and he himself succumbed to an attack at Perpignan. He says that, of its ravages in the Peninsula, no better picture has been given us than that of Johnson in 1771. 'The attack on Carthagera is yet remembered, where the Spaniards from the ramparts saw their invaders destroyed by the hostility of the elements; poisoned by the air and crippled by the dews; where every hour swept away battalions; and in the three days that passed between the descent and the embarkation half an army perished'. In 1783 Masdevall described the lamentable effects of malaria at Lerida, and the devastation it caused among the French troops at Caeceras.

Coming to more recent times, in our wars with the Dutch in Java in 1800, the personnel of the navy engaged there were so affected by the malaria that Edam was described as like a 'charnel house', because of its effects. The ill-fated Walcheren Expedition of 1809 to the Lowlands has often been quoted as an example of the devastating action of malaria upon a British force, although some more recent workers doubt whether the cause was mainly malarial. Of 43,521 men, 18,000 died or were invalided in 3 months, and within 5 months the entire force was destroyed by fever.

This is the disease which contributed very largely to Napoleon's failure in Egypt and Palestine, just as it had played havoc with the forces of the Crusaders in the same country some centuries previously. (It would probably have meted out the same fate to the British forces during Allenby's campaign in Palestine, but for the extensive precautions which were taken to combat its effects.) Our troops in the Crimean War also suffered much from this disease. Macdonald (1923) points out that some of this force occupied practically the same area as did the Macedonian Expeditionary Force in the last war, and suffered from malaria to the same extent. 'In August 1855, from being in extraordinarily fit condition in the previous months, the armies in the Balkan peninsula were suddenly prostrated by disease. Apart from the hospital sick, it was universally admitted that no man in the ranks, though not on the sick list, was capable of active field service'.

Malaria has been a notorious scourge of armies not only in the tropics and the sub-tropics, but also in certain parts of the temperate zone.

Macdonald (1923) notes that 'in armies the debility resulting from malaria causes an immense loss of strength. Exposure, exertion, and fatigue magnified its effects, and the sufferers nearly all came under medical treatment and had to be admitted to hospital'. Cases, which might be able to carry out a certain amount of work under civilian conditions, cannot be placed on duty under field service conditions, except under circumstances of great military urgency. The civilian is usually able to avoid the sudden calls for great exertion which would cause him to break down. The soldier is always liable to such sudden calls, and a force in which a large number of its members will break down under such conditions may seriously endanger the success of the military operations in progress. The requirements of military service aggravate the physical debility, and malaria is consequently much more disastrous in armies than in civil life. In addition to the large numbers which are admitted to hospital, the men who remain on duty gradually deteriorate in fitness for field service.

The mass debility from this disease far exceeded the losses from any other during the Great War. This has been discussed by Macdonald (1923) and by Wenyon (1923) in the Official History of the Great War. On army fronts such

as Macedonia, Palestine, Mesopotamia and East Africa, this disease placed a very serious obstacle in the way of the success of the military operations. The loss of numerical strength and the general deterioration of the troops in the field from this cause were very great. In Macedonia, malaria more than any other single factor was responsible for our impotence on this front.

The incidence of malaria was probably greater in the East African campaign than in any other during the Great War. Here of the recorded hospital admissions, 57 per cent were due to malaria, a rate which was several times heavier even than in Macedonia, while the disability caused by this disease was much greater than these figures show. Between June 1916 and October 1917 there were no less than 107,000 admissions for this disease, while during the whole period of the war, the total admissions for malaria were probably at least a quarter of a million (Keyworth, 1929). Wenyon (1923) reports that in Macedonia during the six most malarious months of 1916 the admission rate for this disease was 237.28 per mille, 277.85 per mille in 1917, and 253.82 per mille in 1918.

Oberlé and Viallette (1923) state that in 1917-18, one-third of the Spanish Army in Morocco was immobilised by malaria. Some 21,000 men spent 318,310 days in hospital. A battalion of 890 men had 286 cases and 32 deaths in one year, and it was held that 50 per cent of the personnel of the army was infected in the first 12 months.

These figures give some idea of the mass debility which malaria may cause in armies or other forces operating in localities where the disease is prevalent. The conditions are really an aggravation of those which are known in civil life as 'tropical aggregation of labour'.

It is evident that such serious debility and deterioration among military forces must place a very heavy additional financial burden upon the administration which has to pay for such an expedition.

India has felt the weight of this burden in military campaigns carried out in malarious areas, during the season when this disease is prevalent. Malaria was responsible in recent years for a very large amount of sickness and disability among the troops operating on the North-west Frontier during the Afghan and Waziristan Campaign of 1919-20. The troops and military police operating in Burma a few years ago were also seriously affected by this disease.

The evidence given above indicates the great additional expenditure which any administration has to bear, whose forces are situated in malarious localities, either under peace or field service conditions, and also the action of this disease in causing a high degree of inefficiency among such forces.

(c) SUMMARY.

The data given above show the numerous ways in which administrations and Governments in malarious zones have to suffer financially because of direct losses from diminished revenue from taxation, from a limitation of interest on capital invested in large undertakings, such as railroads, irrigation schemes, ports, etc., and from expenses needed for the treatment of a sick population, whether official or non-official.

Indirectly such administrations are taxed because of the relatively inefficient or deficient service given by its employees, through the effects of this scourge. In addition to this, in order to obtain effective service, it is often necessary to employ a larger staff and to offer higher rates of pay, higher pensions, better leave conditions, etc., etc., before the proper type of person can be attracted to accept employment.

The greatest indirect loss, which a malarious country like India has to bear, is caused by the obstacles which this disease places in the way of the

full and successful development of her natural resources. If these obstacles were removed, or ameliorated largely, the great increase which would result in the wealth and prosperity of the people would enable larger sums to be placed at the disposal of the administration, and so lead to a greater and more rapid progress towards the attainment of that position among the nations of the world to which her immense natural resources and huge population would appear to entitle India.

(VI) CONCLUSIONS.

The question of the financial and economic losses which malaria causes, both directly and indirectly, to the people of India has been considered from the aspects of the individual, the family and the community, as well as from those of the agriculturist and the industrialist, and of administrations and Governments.

The conclusions arrived at are that this disease almost certainly gives rise to the greatest economic problem with which India has to deal, through its effects on labour efficiency.

The financial losses to the individual and the family alone have been calculated at not less than Rs. 11,000 lakhs, or about £80 million sterling per annum.

Apart from this enormous sum, the financial and economic losses which the effects of this disease cause to the fullest exploitation of the natural resources of the country (agricultural, mineral, etc.), and to the successful development of various kinds of manufacturing industries, are many times greater. While it is not possible to evaluate with any degree of accuracy the immensity of these direct and indirect losses, there is little reason to doubt that they must run into unbelievable millions of pounds sterling each year.

The economic and political progress of all administrations and Governments in India is hampered by the effects of this disease, and, through their action in preventing the normal increase in the prosperity and wealth of the nation, the revenue available for schemes of social and material advancement remains limited.

(To be continued.)

ON THE CAUSES OF MARSH POISON IN INDIA.*

BY

T. E. DEMPSTER.
(*Bengal Medical Service.*)

(1857.)

(To the Editor of the *British and Foreign Medico-Chirurgical Review.*)

SIR,—In the October number of the *Medico-Chirurgical Review* for 1855, you did me the favor to notice the report of a committee (of which I was the sole medical member) on certain sanitary questions connected with canals and canal irrigation in India, and you conclude that notice with the following remarks :—

‘ We cannot conclude this notice of the valuable inquiry carried out by Mr. Dempster and Major (now Colonel) Baker, without expressing a hope that we may again hear from them, and that the above summary of results arrived at by those gentlemen may excite others to institute similar investigations ’.

In answer to this invitation, I now beg to lay before you some very interesting and important additional facts relating to the subject which have recently been brought to my knowledge, and which I hope will be found worthy to be communicated to the profession in England and the colonies, through the medium of your journal.

In autumn last, a fever of an unusually virulent character committed great havoc in a ‘ zilla ’ or district about twelve miles distant from the city of Allyghur, in the upper provinces of the Bengal Presidency. It was of much importance to ascertain, if possible, what was the real nature of this disease. Was it an epidemic, arising from occult atmospheric or other influences which

* This article is reprinted from *The Indian Annals of Medicine*, Vol. V (1858), pp. 293–295.

It was Dempster (1848) in his work on ‘ The Application of the Test of Organic Disease of the Spleen Rate as an easy and certain Method of Detecting Malarious Localities in Hot Climates ’, who first pointed out the value of the spleen rate as a measure of malarial incidence in unhealthy areas in the tropics. As these classical observations were almost forgotten, and the original publications were difficult to obtain, Dempster’s papers were reprinted in the *Records of the Malaria Survey of India*, Vol. I, No. 2, pp. 1–69. Recently we have been fortunate enough to discover a later note by Dempster on the same subject. This article, apart from its historical interest, records a number of observations upon the relationship between flooding and epidemic malaria, and these alone make the note worthy of preservation from oblivion. (*Editor.*)

can neither be foreseen nor counteracted by any means yet at our disposal? Or was it a pure endemic, depending on local, and perhaps easily remediable causes? These were obviously questions of much practical import to the inhabitants of the district under consideration.

The fever was said by some to be contagious, but mention was also made of a 'nulla' (the Korrum) having overflowed its banks, and it was determined to apply my spleen test for malarious fever. The examinations were commenced nine miles from the 'nulla', and carefully continued up to it. It was found that there had been an unusual amount of fever all over the district, and several cases of diseased spleen were brought up for advice. For the test purposes, however, these were not counted, but the *bystanders examined just as they came to hand*. Till a village was reached three miles from the 'nulla', and to some extent influenced by its inundation, no case of spleen was found by an examination so conducted. In this village, however, 60 per cent had spleen.

On reaching the 'nulla' this rate kept up, and in one village (Chandous) which had suffered most from fever, 80 per cent of the remaining inhabitants were found afflicted with diseased spleen! This at once indicated a severe form of intermittent fever, and many other circumstances combined to confirm this belief, viz. :—

1. Three escape channels from the New Ganges Canal enter the 'nulla' above the village of Chandous, and fill it to *overflowing* even in the cold or dry season, for a distance of some ten miles. During the periodical rainy season this prevented the water from finding an escape, and the country was consequently greatly flooded.

2. The villages *above* Chandous suffered to no unusual extent from fever.

3. The villages *on* the 'nulla', or inundations formed by it, have suffered out of all proportion to the rest of the country.

4. At Chandous itself, where the canal water enters, 3,000 people out of a population of 3,600 have been carried off. This mortality was greatly assisted by the flood entering a 'jheel' or shallow lake, and thus increasing the malarious surface to a great extent.

5. The side or sides *nearest* the 'nulla' of other large villages opposite a less extent of inundation, suffered most. The centres and other sides seem to have been protected. This suffering of particular sides of villages was distinctly seen from the tops of the 'zemindar's' houses, as on those sides the houses of the villages were observed to be in ruins.

6. Smaller villages close by were overwhelmed by the malaria—that is, were, with here and there an exception, a mass of roofless houses.

7. At about ten miles below Chandous the bed of the 'nulla' is larger, or the flood had expended itself in spreading right and left, and there there was no unusual fever.

8. In the district generally people died in from five to fifteen days from fever last year. At the village three miles from the 'nulla' (where 60 per cent of spleen disease was found) they died in three days. *On* the 'nulla' (where the percentage of spleen disease among the survivors amounted to eighty) they died in twenty-four hours during the *ague* or cold stage of the fever.

Some other points of interest were observed, but enough has been stated to prove the accuracy of the test I proposed, and its practical utility in leading at once to the right conclusions in investigations of this nature, in certain provinces of our Indian dominions.

In my appendix to the Canal Sanitary Report I showed why the large military station of Kurnaul had been abandoned.

For a great many years a canal had flowed in the immediate vicinity of that large cantonment, and yet, according to Colonel Tulloh's tables, it ranked in point of salubrity for European troops second or third among all the military stations of the Bengal Presidency. This canal (the Delhi) was an old Mahomedan's work, and only re-opened and improved by us. It occupies the bed of a large 'nulla'—the natural drainage channel of that part of the country—and follows its most tortuous windings. So long as the *highest* level of the water in this canal was *below* the lowest ground in the neighbourhood of the cantonment, it still performed its office of a natural drain; but when—with the most humane and benevolent intentions—the level of the water in the canal was raised so high that it could deliver water *on* the low ground opposite Kurnaul for the purposes of irrigation, it is obvious that no more drainage could escape by that channel. No other existed; and when the periodical rains came, all the low ground in the vicinity of the cantonment became an extensive swamp, and the troops, European and native, were universally prostrated by fevers of the most virulent type.

A remedy—an expensive one, it is true—might have been applied; but Kurnaul was no longer the important military position it was once esteemed, and Lord Ellenborough abolished it altogether as a cantonment for European troops.

Precisely the same thing has now occurred in the case above related. The Great Ganges Canal is itself constructed on proper principles. It passes along the '*watershed line*' of the country through which it flows, and can never interfere with its natural drainage, whatever amount of water it may carry down to fertilise the land. But it appears it lately became necessary to expose a part of the bottom of this new canal, and this was effected by turning three escape channels into the 'Kurram nulla', by which it was filled to *overflowing*, and no other drain for the country being provided, all the low ground in the neighbourhood was swamped during and after the periodical rainy season.

The fatal results of this mistake have been fully detailed above. The whole of this subject has an important bearing on all questions relating to the medical topography of a great part of the Bengal Presidency.

In conclusion, I would respectfully urge on the Indian Government the necessity of strictly enforcing the limits of the sanitary zones recommended by our committee, and of prohibiting canal irrigation within five miles of all large military stations occupied by European troops. If the principles on which these zones were defined be correct, their strict observance becomes of greater importance than ever, now that so large and permanent an addition is about to be made to our European troops in Bengal.

I remain, yours faithfully,

T. E. DEMPSTER,
Bengal Medical Service.

31st August, 1857.

FAILURE TO PRODUCE INFECTION IN TWO SPECIES OF
THE LOWER ORIENTAL MONKEYS BY THE INJECTION
OF MALARIAL SPOROZOITES FROM NATURALLY
INFECTED ANOPHELINES.

BY

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AND

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[19th October, 1935.]

UNSUCCESSFUL attempts to infect a specimen of *Silenus rhesus*, the common brown monkey of northern India, with living sporozoites from the glands of wild-caught anophelines were recorded by Majid (1933). This author made the gland dissections in normal saline solution, and subsequently inoculated the sporozoite suspension in this fluid into a normal monkey, by the subcutaneous or by the intraperitoneal route. Taliaferro and Taliaferro (1934) claim to have succeeded in transmitting the human malarial parasite, *P. falciparum*, to one of the lower monkeys of America (*Alouatta* sp.). The success of the latter experiments, where many similar attempts have failed in other hands, may be due to the use of a different species of monkey, or more probably to the fact that very large doses of infected human blood were injected. The literature on this subject has been summarised by Majid (1933) and by Taliaferro and Taliaferro (1934).

During the course of our routine duties in these laboratories, a large number of anopheline mosquitoes was dissected and examined for malarial infection. These mosquitoes were received from Quetta, Baluchistan, where they had been captured in nature. Many of them showed extremely heavy infections of the salivary glands with sporozoites, and there is little reason to doubt that these were of human origin.

In view of the successful results recorded by Taliaferro and Taliaferro (1934), after the inoculation of large numbers of malaria parasites from human

patients into monkeys, it was decided to try whether very large doses of motile sporozoites from the salivary glands of naturally infected mosquitoes would give equally successful results.

TECHNIQUE AND MATERIAL USED.

All the mosquito dissections were carried out in Ringer's solution. Only those glands which showed extremely heavy infections with sporozoites were used for inoculation. In many instances, the infections were so heavy that the sporozoites appeared closely packed together in the microscopic field.

The infected glands were completely disintegrated by pressure upon the cover-glass, more dissecting fluid added and the resultant suspension taken up into a small glass hypodermic syringe. As short an interval of time as possible was allowed to elapse between dissection and injection. The injections were made on each occasion by both the intravenous and the subcutaneous routes, the dosage by each route being approximately equal. Two monkeys were used (one *S. irus* and the other *S. rhesus*), and each of these received repeated injections of sporozoites.

It has been suggested by Sinton and Mulligan (1933) that a certain degree of maturation of sporozoites may be necessary before these become infective. In our experiments we have every reason to believe that each of the two experimental animals must have received injections of sporozoites which had had the opportunity of maturing. This is suggested by the following facts:— (i) it is highly improbable that so many infected insects, caught in nature, would all have immature sporozoites, (ii) the dissections were made from 3 to 7 days after capture, and (iii) in many instances, oocysts were no longer present in the stomach, indicating that all these had reached maturity, ruptured and disappeared.

PROTOCOLS OF EXPERIMENTS AND THE RESULTS.

(1) Monkey No. 572, *Silenus irus*. This adolescent monkey had been under observation for about 16 months, and no evidence of natural infection was detected*.

20-8-35. Injected† with motile sporozoites obtained from the salivary glands of

1 specimen of *A. superpictus* and

1 specimen of *A. stephensi*.

29-8-35. Injected with motile sporozoites obtained from the salivary glands of

2 specimens of *A. superpictus*.

13-9-35. Injected with motile sporozoites from the salivary glands of

1 specimen of *A. superpictus*.

* The blood of this animal was subjected to weekly blood examinations for the whole period of observation prior to inoculation, so as to exclude the presence of any natural infection. The absence of such infection during this long interval also gave an opportunity for any acquired immunity to diminish.

† In all these experiments, approximately equal parts of each sporozoite suspension were given by each of the intravenous and subcutaneous routes.

14-9-35. Injected with motile sporozoites from the salivary glands of 3 specimens of *A. superpictus*.

Result.—This animal was inoculated by the intravenous and by the subcutaneous route with the very heavily infected salivary glands of 8 anophelines caught in nature. Although this animal has been observed by daily thick-film blood examinations for 64 days, no detectable infection has developed.

(2) Monkey No. 262 (*S. rhesus*). This young normal* monkey was also inoculated by both the intravenous and the subcutaneous routes with sporozoites.

17-8-35. Injected with motile sporozoites from the salivary glands of 1 specimen of *A. superpictus*.

20-8-35. Injected with motile sporozoites from the salivary glands of 1 specimen of *A. superpictus* and 1 specimen of *A. culicifacies*.

30-8-35. Injected with motile sporozoites from the salivary glands of 2 specimens of *A. superpictus*.

13-9-35. Injected with motile sporozoites from the salivary glands of 4 specimens of *A. culicifacies*.

Result.—This animal was inoculated by the intravenous and by the subcutaneous route with the very heavily infected salivary glands of 9 different anophelines caught in nature. Although this animal was observed by daily thick-film blood examinations for 65 days, no detectable infection developed.

SUMMARY.

Very large numbers of motile sporozoites, many or most of which were presumably mature and infective, from the salivary glands of 17 different anophelines caught in nature were suspended in Ringer's solution and inoculated into two Oriental monkeys of the genus *Silenus*. These sporozoites were almost certainly all of human origin, in as much as they were found in anophelines collected during the height of the malaria season in a highly malarious area.

Although these two animals (*S. irus* and *S. rhesus*) were examined carefully by the thick-film method daily for 64 and 65 days, respectively, after the primary inoculation, no malarial infection could be detected, in spite of the fact that enormous numbers of motile sporozoites, presumably of human origin, were inoculated.

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* This species of monkey in northern India has been shown to be very susceptible to infection with the three common species of simian *Plasmodia* found in the East. These animals have never been found infected in nature, so have no chance of having any acquired immunity.

ANTI-LARVAL OIL APPLICATION BY A 'ONE MAN BRUSHING METHOD.

BY

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[19th October, 1935.]

INTRODUCTION.

QUAIFE (1931), working on an economical but efficient means for anti-larval control of malaria, described the brushing method of oil application as a modification of the previously used method of applying oil with a small mop. In describing the method he says, 'two men are necessary. One takes the oil and a small mop, and having dipped this into the oil, sprinkles the water, walking forward four paces, and then dips again. The other man lightly brushes the oil and water to and fro on the edges.....A fine mixture results and an even continuous film of oil is left on the water'.

After much experience with the method, Quaife (1934) has shown that the larvicidal action of oil when applied in this way justifies its use when directed against *A. maculatus* which breeds in the margins of streams, drains and seepages in Malaya. Other workers in Malaya who have adopted the brushing method against *A. maculatus* have confirmed its efficacy and economy.

During 1935, Quaife's brushing method was used for oil application to the streams and seepages of one control scheme in Assam, in order to determine its practicability and efficacy when directed as an anti-larval measure against *A. minimus* the local vector. The method proved both practical and economical, by killing the larvæ and by reducing the amounts of oil required for the given stream yardage or seepage surface.

The purport of this paper is to describe a method by which the expenditure for labour can be reduced by making it possible to apply effectively the brushing method of oil application by the efforts of one man instead of two men as previously required.

DESCRIPTION OF THE ADAPTED SPRAYER.

In seeking for a suitable means by which the oil could be easily carried by one man and be injected into the brush in the required quantities, but would

not hinder the necessary motions for efficient brushing, it was found that, after making slight alterations, the 'Knapsack Solo Sprayer' fulfilled these requirements (Plate VI).

The oil delivery of the Solo Sprayer is actuated, on the ball valve principle, by a brass pump having two handles and an original thrust of about 12 inches which delivers the oil to the spraying nozzle. The modification merely comprises the removal of the distal hand grip, shortening the pump thrust to six inches by attaching a longer hand grip to the male part so that a 24-inch bamboo brush can be attached to the distal end of this hand grip. The brush is secured to the handle by means of two motor car hose-pipe connection fittings. The oil delivery pipe extends one inch beyond the distal hand grip into the centre of the brush and has fitted to it the largest size spraying nozzle. The bamboo brush can be easily replaced as required.

METHOD OF OIL APPLICATION.

The labour of one man only is required for oil application with the modified Solo Sprayer.

The method of applying oil is much the same as that described by Quaife (1931). The man starts at the lower end of the stream or drain and, if it is not more than one yard wide, he brushes the oil from an acute angle on to first one, and then the other bank of the stream, always working upstream. For wider streams, one bank should be treated at a time, working from downstream upwards on first one and then the other bank. The oil is delivered into the brush by a to-and-fro pumping motion of the handle as brushing is being carried out, and the amount of oil required is gauged by the film being laid. By brushing the water at an acute angle towards the bank, oil is thrown high on to the edges of the stream or drain and thus covers the soil with a thin film of oil which slowly trickles back into the water channel. Where seepages occur along the side of a water channel, these should be thoroughly brushed.

This method of oiling does not kill the grass to which *A. minimus* larvæ are prone to attach themselves and it is necessary therefore that the edges of the stream and drains be kept free from vegetation but, as remarked by Quaife (1931), 'It is cheaper to scrape the grass by hand labour than to use expensive oil to kill it'.

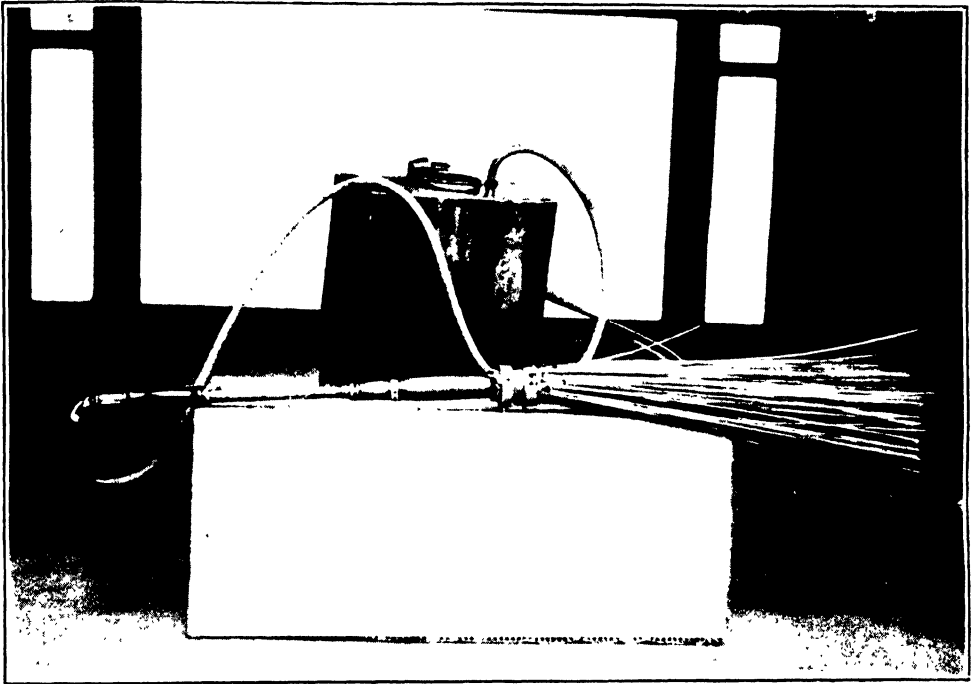
CONCLUSION.

A method of, and the apparatus for, applying anti-larval oil by the brushing method with the labour of one man, are described.

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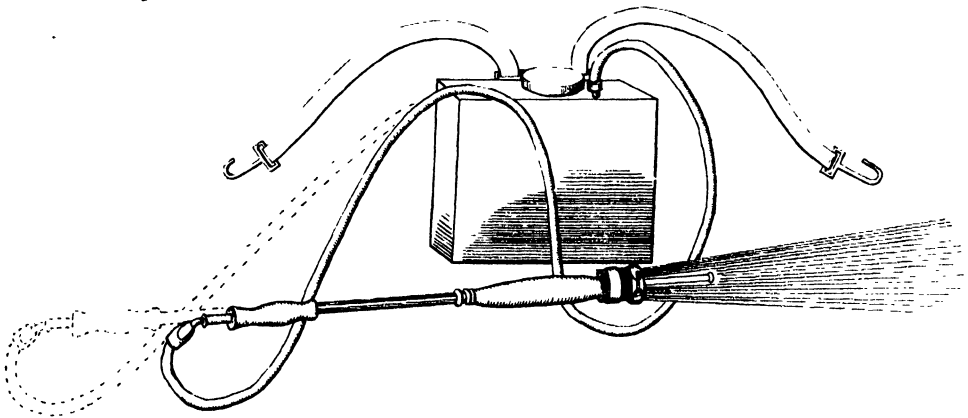
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PLATE VI.



Solo Sprayer

Adapted for Brushing



STUDIES IN IMMUNITY IN MALARIA.

Part V.

THE NATURE OF THE TOLERANCE SHOWN BY *SILENUS IRUS* TO INFECTION WITH *PLASMODIUM KNOWLESI*.

BY

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It has been recorded by many workers that the clinical characteristics of both natural and inoculation infections of *P. knowlesi* in laboratory specimens of *S. irus*, are the very mild nature, or the absence, of any symptoms in the infected animal. These findings are in very marked contrast to the extremely severe and fatal results which almost invariably follow inoculation of this infection into another species of the same genus, namely *S. rhesus* (*vide* Napier and Campbell, 1932; Knowles and Das Gupta, 1932; Sinton and Mulligan, 1933*b*; etc.).

From these reports it is evident that, while *S. irus* is not naturally *immune* to infection with *P. knowlesi*, specimens of the type used in most laboratories show a very considerable degree of *tolerance* to the pathogenic effects of the infection. Much uncertainty exists as to whether this tolerance is to be considered as due to some natural or innate factor, or to an acquired immunity resulting from a prior or a latent infection, or to a combination of these factors*.

While there is much epidemiological evidence which suggests that certain races of man have naturally a higher degree of tolerance to the effects of malarial infection, or the power to develop a tolerance more rapidly and to a greater degree, than occurs in some other races, yet there is no experimental proof to confirm this belief. The occurrence of a high degree of tolerance in *S. irus*, as compared with *S. rhesus*, appeared likely to provide some evidence to aid in the solution of this problem.

* This subject has been discussed at some length by Sinton and Mulligan (1933*c*) (pp. 784-788).

Obviously, the most certain method of arriving at a correct understanding of the problem would be to experiment with animals bred and living under conditions which precluded all possibility of malarial infection, and so of any acquired tolerance. So far we have been unable to obtain animals of this nature. The only specimen of *S. irus* which has been born in our animal houses unfortunately died when about 3 months old, and before it had reached a suitable age for the contemplated experiment. It has, therefore, been necessary to tackle the problem in a different manner.

The results of some of the earlier observations recorded in literature, suggest that the tolerance observed in *S. irus* may be of an acquired nature :—

(i) Blanchard and Langeron (1912) report that the experimental inoculation of 2 specimens of *S. irus* with blood from a natural infection* in another animal of the same species, resulted in one case in a very acute infection resembling a pernicious attack in human malaria. In a second paper (Blanchard and Langeron, 1913), they record that 1 out of 7 animals of the same species died of an acute infection, when inoculated with their strain of simian parasite*, while the others survived for many weeks or months with chronic infections. These appear to be the only records of very acute attacks developing in apparently normal specimens of *S. irus* after infection with a parasite which was possibly *P. knowlesi*.

The animal which developed the acute infection in the first experiments of Blanchard and Langeron had been under observation for more than a year in Paris, and had shown no signs of malaria during that period. This finding suggests that the observed tolerance in this species of monkey may be mainly acquired, and that during the apparently infection-free observation period any previously acquired tolerance had disappeared.

(ii) The common occurrence of natural infections of malaria in *S. irus* makes it very possible that many of the animals available for experimental purposes had had the opportunity of acquiring a considerable degree of tolerance before they came under observation (*vide* Sinton and Mulligan, 1933c). Even although such animals may appear to be uninfected at the time, it is often very difficult to exclude the possibility that a high degree of acquired tolerance is being maintained by an undetected latent or sub-patent infection, as has been pointed out by Sinton and Mulligan (1933c) and by Knowles and Das Gupta (1934).

(iii) The results of infection with *P. knowlesi* in *S. irus* bear a close resemblance to those reported by Mulligan and Sinton (1933, 1933a) and by Sinton and Harbhagwan (1935), in specimens of *S. rhesus* which had acquired a high degree of tolerance as the result of multiple superinfections. This suggests that the two conditions may be similar.

While the observations discussed above suggest that the tolerance in *S. irus* may be an acquired one, there are other recorded data which are more in favour of the view that much of this tolerance may be a natural characteristic.

* Blanchard and Langeron (1912, 1913) identified the parasite responsible for these infections as *P. cynomolgi*. On the other hand, Sinton and Mulligan (1933c), from a study of the data and illustrations given by these workers, consider that they were more probably dealing with a mixed infection, of which one parasite was *P. knowlesi*.

(i) It has been found that many species of the lower monkeys apparently possess an absolute natural *immunity* to infection with human malarial parasites. This may only represent a higher degree of the type of *tolerance* exhibited by *S. irus* to infections with *P. knowlesi*.

(ii) The fact that other species of the lower monkeys vary considerably in the severity of their reaction to infection with *P. knowlesi*, suggests that a natural tolerance of greater or less efficacy may be present in some species. Some monkeys such as *Pygathrix entellus* and *Py. schistaceus* have been found to be very susceptible, while *S. sinicus* appears to be intermediate in susceptibility between *S. rhesus* and *S. irus*.

(iii) The work of Mulligan and Sinton (1933, 1933a) has shown that the acquired tolerance of *S. rhesus* to a strain of *P. knowlesi* is usually only effective against the same strain of this parasite. Unless the tolerance acquired by *S. irus* is of a different character from that observed in *S. rhesus*, one would not have expected that so large a proportion of the specimens of *S. irus*, which have been experimentally infected with *P. knowlesi*, would have possessed such a high degree of tolerance to all the different strains which must have been used by various workers*.

(iv) It has been shown by Knowles and Das Gupta (1932) that the anthropoid ape, *Hylobates hoolock*, is almost insusceptible to infection with *P. knowlesi*. The same workers have found that, while human patients can be infected with this parasite, the clinical manifestations of the disease are relatively mild and tend to spontaneous cure. There appears to be little or no chance that these patients could have suffered from a prior infection with *P. knowlesi*, and so have developed any acquired tolerance. These results suggest either that man possesses a degree of natural tolerance to infection with *P. knowlesi*, or that previous infection with human plasmodia may cause a cross immunity. There is, however, no evidence that the latter occurs to any marked degree with either human or simian parasites.

It is obvious that no definite conclusions can be arrived at as to the origin of the tolerance exhibited by *S. irus* to *P. knowlesi*, from the data given above.

The observed tolerance of *S. irus* to the pathogenic effects of infection with *P. knowlesi* can be broken down by splenectomy. After this operation, the infection behaves in a manner very similar to that seen in the severe and fatal disease recorded in *S. rhesus* (Blanchard and Langeron†, 1913; Sinton and Mulligan, 1933b; Krishnan, Smith and Lal, 1933).

While such an abnormal procedure may be of value in detecting or activating a latent or sub-patent infection of *P. knowlesi* in *S. irus*, it gives little or no information as to whether the tolerance observed in this animal is of natural or acquired origin. In addition, animals, from which the major

* This is especially the case with the experiments reported by Malamos (1934). This worker used the K₁ strain of *P. knowlesi*, isolated by us from experimental animals in India, to cause infections in specimens of *S. irus* obtained in Europe. This makes it very unlikely that the strain of parasite and the specimens of monkey would have come from the same locality, and so have given the animals an opportunity of acquiring a homologous tolerance.

† Vide footnote, p. 502.

portion of the reticulo-endothelial system has been recently removed, can no longer be considered as reacting to infection in the same manner as normal animals*. It was, therefore, decided to determine whether the tolerance of specimens of *S. irus* was diminished as the result of prolonged freedom from malarial infection, and whether it could be broken down by massive doses of single and multiple strains of *P. knowlesi*. It was hoped that, by these means, some light might be obtained on the problem.

RESULTS OF EXPERIMENTAL WORK.

(1) INOCULATION OF A SMALL DOSAGE OF A SINGLE STRAIN OF *P. KNOWLESI* INTO NON-INFECTED SPECIMENS OF *S. IRUS*.

(A) EFFECTS OF A SMALL DOSAGE OF PARASITES IN ANIMALS POSSIBLY POSSESSING SOME RESIDUAL ACQUIRED TOLERANCE.

Experiment (a).

Five young specimens of *S. irus*, purchased in Calcutta, were tested for the presence of malarial infection by (a) iso-diagnosis, (b) protein shock, and (c) repeated blood examinations by the thick-film method over an average period of 30 days. By none of these methods could any evidence of infection be detected. Each of the animals was then inoculated intraperitoneally† with 0.25 c.c. blood taken from infected specimens of *S. rhesus*. The strains used in the different animals were C, K₁ and K_a.

Although all the animals developed infections, in no case were these accompanied by noticeable clinical symptoms, nor did the parasite prevalence ever rise to a relatively high level.

(B) EFFECTS OF A SMALL DOSAGE OF PARASITES ON ANIMALS PROBABLY POSSESSING LITTLE OR NO RESIDUAL ACQUIRED TOLERANCE.

While no evidence could be found that any of the specimens used in the previous experiments were infected at the time of inoculation, there was always a possibility that some of them might still have retained a certain degree of residual acquired tolerance, as the result of a previous infection which had been radically cured. As there is some evidence to suggest that the tolerance acquired in this way usually diminishes comparatively rapidly in its efficacy after cure, the animals used in the following experiments were kept under observation for many months before inoculation. This was done, not only to exclude as far as possible the presence of a latent infection, but also to allow any previously acquired tolerance to fall to a low level. In some cases the animals were also given a thorough course of anti-malarial treatment to ensure that no sub-patent infection existed to account for any maintenance of tolerance at a high level.

* *Vide* Addendum (pp. 519-521).

† All inoculations in the experiments reported in this paper were by this route unless otherwise stated.

Experiment (b). Monkey No. 120 (*S. irus*).

This animal was kept under observation for nearly 22 months before being inoculated with *P. knowlesi*.

History of period of observation. During the first 11 months, weekly blood examinations by the thick-film method failed to reveal any parasites. The animal was then given protein shock by the intravenous injection of 2 c.c. of human blood. Daily blood examinations for about 4 weeks after this failed to show any parasites, nor did a normal specimen of *S. rhesus* inoculated with blood 5 days after the protein shock develop an infection. A similar inoculation into another specimen of *S. rhesus* also failed to produce infection, when made 5 weeks later. Weekly blood examinations, continued up to nearly the end of the 19th month of observation, showed no parasites. The animal was then given a thorough course of anti-malarial treatment* during the next month. Weekly blood examinations up to the end of the 22nd month still gave negative results. As a result of this prolonged period of observation, it was concluded that this animal had been free from infection for at least 22 months, or nearly two years, and this was thought a sufficiently long period to allow any acquired immunity to drop to a low level.

History of infection. Inoculated intraperitoneally with blood taken from Monkey No. 241 (*S. rhesus*) infected with strain C of *P. knowlesi*. Parasites detected on 5th day in scanty numbers, which continued until 10th day, then slight increase occurred for 2 days. Parasites present daily in scanty or very scanty numbers till 36th day. Parasites not found again up to 150th day of observation, except for very scanty numbers on 39th, 40th, 61st and 74th days, and in extremely scanty numbers on 48th, 49th and 140th days, in spite of daily blood examinations.

Result.—A very mild parasitic attack without any noticeable clinical symptoms, and which recovered spontaneously. No marked parasitic relapses were observed during an observation period lasting up to 150 days after inoculation.

Experiment (c). Monkey No. 123 (*S. irus*).

This animal was also under observation for about 22 months before being inoculated.

History of period of observation. The duration of the period of observation, and the methods employed to detect and eliminate a latent infection, were exactly similar to those employed in the last experiment.

History of infection. Inoculated intraperitoneally with blood taken from Monkey No. 240 (*S. rhesus*) infected with K, strain of *P. knowlesi*. Parasites not detected by daily blood examinations until 15th day. Afterwards found in very scanty numbers at daily examinations up to 45th day. No parasites were then detected from 46th to 91st days, when the animal died of an intercurrent disease.

Result.—An extremely mild parasitic infection without any clinical manifestations. No parasitic relapses during a period of observation lasting up to 90th day.

Experiment (d). Monkey No. 101 (*S. irus*).

This animal was under observation for 13 months before being inoculated.

History of period of observation. Weekly blood examinations for 13 months revealed no parasites, nor did a normal specimen of *S. rhesus* injected with the blood of this monkey develop an infection.

* The course of treatment consisted of—0·13 gm. of quinine sulphate in solution daily by the mouth for 1 week; during the next week 0·005 gm. plasmoquine was injected intramuscularly each day; after an interval of 10 days, 0·025 gm. atabrin was given daily by the mouth for one week. This completed the treatment.

History of infection. Inoculated intraperitoneally with blood taken from Monkey No. 551 (*S. rhesus*) infected with strain K₄ of *P. knowlesi*. Parasites detected in extremely scanty numbers on 3rd day. A distinct parasitic attack, without clinical symptoms, developed from 7th to 9th days. The infection then became scanty for 4 days, and very scanty up to 18th day. No parasites were seen from 19th to 21st days, while on 22nd day they were again very scanty. The parasites continued to increase and there was a slight parasitic relapse with its maximum from 26th to 30th days. A very scanty infection then developed and continued in the same state, or less intensely, up to about 80th day. No parasites could be detected from the latter day up to 90th day, after which an extremely scanty infection developed for 6 days followed by an absence of parasites for 11 days. [The later history of this animal, which was superinfected on 99th day, is given in Experiment (h)].

Result.—A distinct parasitic attack developed without clinical symptoms, and this was followed by a slight parasitic relapse*.

(C) DISCUSSION OF EFFECTS OF A SMALL DOSAGE OF A SINGLE STRAIN OF *P. KNOWLESI* UPON THE TOLERANCE OF *S. IRUS*.

While no evidence of malaria infection could be detected in the five animals in Experiment (a), the possibility of the existence of some residual acquired tolerance could not be ruled out entirely. Such a condition may have persisted through the comparatively short periods during which the animals were under observation before inoculation.

That the tolerance shown by these animals could not be entirely of an acquired nature, is suggested by the facts that several different strains of *P. knowlesi* were used, and that the animals were purchased at different times and thus probably originated from different localities. In such circumstances, one would not expect that all these animals would have had opportunities to acquire and retain homologous tolerance to the varied strains of parasite inoculated.

To eliminate as far as was practicable the effects of any existing acquired tolerance, three animals were kept under careful observation for periods lasting for 13 to 22 months before inoculation, during which time no evidence of malarial infection could be detected. In spite of this the results of inoculation showed that a high degree of tolerance still existed in these animals†.

The results of Experiments (a), (b), (c) and (d) would seem to be explicable only on the following hypotheses:—

(1) that any tolerance once acquired may last for a very long period, even in the absence of a sub-patent or latent infection. Such a view, however, would not be in keeping with the present belief that malarial immunity is comparatively short-lived, and

(2) that the tolerance observed in *S. irus* is mainly due to natural factors and not to acquired ones.

* The occurrence of a parasitic relapse is interesting, for it is this K₄ strain which has been found to show marked relapsing properties in *S. rhesus* [vide Mulligan and Sinton (1933a) and Sinton and Harbhagwan (1935)].

† The animal in Experiment (j) (vide Addendum) had a natural infection with strain K₃, which is heterologous to strain K₄, so presumably this animal was not infected with the latter strain when observation commenced. Although this monkey was kept under observation for 39 months, it still showed a tolerance to superinfection with strain K₄ at the end of that time.

(2) INOCULATION OF LARGE DOSES OF A SINGLE STRAIN OF *P. KNOWLESI* INTO NON-INFECTED SPECIMENS OF *S. IRUS*.

As the previous experiments had shown that our specimens of *S. irus* still retained a high degree of tolerance to infection with *P. knowlesi*, even after being free from infection for very long periods, it was decided to try whether this observed tolerance could be broken down, or markedly diminished, by a heavy dosage with a single strain of this Plasmodium.

(A) EFFECTS OF MULTIPLE CONSECUTIVE INFECTIONS WITH A SINGLE STRAIN.

Experiment (e). Monkey No. 571 (*S. irus*).

As shown in the following protocol, no evidence of infection could be detected in this animal during an observation period of 300 days before inoculation. It was then given a daily dose of blood from animals infected with strain K₂ of *P. knowlesi* on 6 consecutive days.

History of period of observation. Inoculation of the blood of this animal into a normal specimen of *S. rhesus* failed to produce an infection, nor could any parasites be detected at weekly thick-film blood examinations during a period of 6 months. At the end of this time it was given a course of anti-malarial treatment similar to that described in Experiment (b). The weekly blood examinations were continued with negative results up to the end of about 10 months.

History of infection. This animal was then inoculated intraperitoneally on six occasions with strain K₂ of *P. knowlesi* from three different monkeys in the doses indicated—

1st day from Monkey No. 559	1 million parasites.
2nd " " " " 559	10 " "
3rd " " " " 559	1 " "
4th " " " " 560	22½ " "
5th " " " " 586	½ " "
6th " " " " 559	1 " "

TOTAL IN 6 DAYS	..	36 million parasites.
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Daily blood examinations revealed parasites in extremely scanty numbers on 11th day. These increased and were present in scanty numbers from 15th to 25th days, after which they became very scanty up to 41st day. Daily examinations from this time until 70th day showed no parasites, except extremely scanty ones on 47th, 48th and 68th days.

Result.—A very mild parasitic attack lasting about a month with no noticeable clinical manifestations.

(B) EFFECTS OF A SINGLE LARGE DOSE OF ONE STRAIN OF PARASITE.

As a heavy dosage of a single strain of parasite spread over 6 consecutive days, failed to have any marked effect upon the observed tolerance of *S. irus*, it was decided to test the effects of a heavy dosage of parasites given at the same time by several different routes of inoculation.

Experiment (f). Monkey No. 584 (*S. irus*).

History of period of observation. Seven daily blood examinations failed to reveal parasites, nor did later ones made at weekly intervals for one month show any infection. The animal was then give daily doses of 0.025 gm. atebirin orally for one week, and observed by daily blood examinations for another month, making a total of 65 days.

History of infection. The following doses of strain C of *P. knowlesi* from Monkey No. 244 (*S. rhesus*) were given at the same time:—(i) 14½ million parasites intravenously, (ii) 14½ million parasites intraperitoneally, and (iii) 14½ million parasites subcutaneously, a total of about 43 million.*

Very scanty parasites were detected in the blood of the recipient animal 5 minutes, 90 minutes and 5 hours after the injections. They continued in very scanty numbers for the next 2 days, after which an infection showing scanty parasites was present daily up to 29th day. The infection was then found to be very scanty until 42nd day. Weekly blood examinations made up to 230th day showed no parasites except for a few on 67th, 68th, 69th, 73rd and 120th days.

Result.—A mild parasitic infection with no noticeable clinical symptoms was detected immediately after the inoculations. This continued for about 6 weeks after which parasites could only be detected at very rare intervals.

(C) DISCUSSION OF EFFECTS OF LARGE DOSES OF A SINGLE STRAIN OF *P. KNOWLESI* UPON THE TOLERANCE OF *S. IRUS*.

The results of Experiments (e) and (f) show that, under the conditions present, the tolerance exhibited by *S. irus* to infection with *P. knowlesi* was not broken down by a large dosage of parasites. This result was observed whether the dose was given on one day by three different routes, or whether it was spread over 6 consecutive days.

These findings help to support the view that the tolerance shown by this species of monkey is dependent mainly upon natural characteristics possessed by the animal.

(3) INOCULATION OF MULTIPLE STRAINS OF *P. KNOWLESI* INTO APPARENTLY NON-INFECTED SPECIMENS OF *S. IRUS*.

As the results obtained by the inoculation of a single strain of *P. knowlesi*, in varying doses and by varying routes, had failed to break down the tolerance of *S. irus*, it was decided to try the results of inoculations with multiple strains.

While it has been pointed out above that it was improbable that all the monkeys inoculated could possess a homologous acquired tolerance to the various strains of *P. knowlesi* used, such a condition could not be excluded with any degree of certainty. To obtain more precise evidence on this point, some of the animals used in the following experiments were inoculated with as many as 7 different strains of *P. knowlesi*, and these strains have been shown by Mulligan and Sinton (1933, 1933a) and by Sinton and Harbhagwan (1935) to be heterologous in their immunological properties. It was hoped in this way that, if the tolerance were an acquired one, at least some of the strains which had been collected at different times and from different batches of naturally infected monkeys during a period of 3 years, would prove to be heterologous in respect to any acquired tolerance present.

* In making these injections, 1½ c.c. of blood was withdrawn from the infected animal into a syringe containing 1½ c.c. of citrate saline solution. The mixture was well shaken, and 1 c.c. of it was at once injected by each of the routes mentioned.

(A) INOCULATIONS FROM MONKEYS INFECTED WITH MULTIPLE STRAINS.

Experiment (g). Monkey No. 573 (*S. irus*).

This animal was inoculated intraperitoneally on the same day with blood taken from three different specimens of *S. rhesus*, which had each been infected previously with 7 different strains of *P. knowlesi*, as well as with *P. cynomolgi*.

History of period of observation. The blood of this monkey was examined on 8 occasions during an observation period of 7 weeks with negative results. Its blood was also inoculated into a normal specimen of *S. rhesus* without causing an infection.

History of infection. The animal was inoculated on the same day with 0.25 c.c. of blood from each of the following animals (*S. rhesus*)*:—

- (i) Monkey No. 7. Infected at various times during a period of 738 days with strains C (twice), K₁, K₂, K₃, K₄, K₅ and K₆ of *P. knowlesi*, and also with *P. cynomolgi*.
- (ii) Monkey No. 29. Infected at various times during a period of 666 days with the same strains and species in the same order.
- (iii) Monkey No. 80. Infected at various times during a period of 564 days with strains K₁, K₂, C, K₃, K₄, K₅ and K₆ of *P. knowlesi* and also with *P. cynomolgi*.

Parasites were detected in very scanty numbers on 5th day. They increased rapidly and a definite parasitic attack due to *P. knowlesi*, but without clinical symptoms, occurred from 7th to 11th days. These parasites then decreased to very scanty numbers to be succeeded by a moderately severe parasitic attack of *P. cynomolgi* lasting from 18th to 25th days, but without any clinical manifestations. The same parasite was seen in scanty numbers for about a month longer, when the infection changed to a scanty or very scanty mixed one of both species of *Plasmodium* from 51st to 88th days, with *P. knowlesi* predominating. From this time to about 100th day very scanty parasites, mostly *P. cynomolgi*, were found daily. Later weekly blood examinations for about 7½ months (up to 322nd day) were often negative, and if parasites were detected they were *P. cynomolgi* in scanty or very scanty numbers. At the end of this period the animal was treated in connection with another experiment.

Result.—A distinct parasitic attack due to *P. knowlesi* occurred, from which recovery was spontaneous. This was followed by a similar attack due to *P. cynomolgi*. Neither of these attacks gave rise to noticeable clinical symptoms. They were succeeded by a mixed infection of both species of parasite. Later a low grade infection of *P. cynomolgi* persisted for about 7 months.

Experiment (h). Monkey No. 101 (*S. irus*).

The animal used in Experiment (d) had developed a chronic infection with strain K₄ of *P. knowlesi*, so it was decided to try whether a distinct parasitic relapse could be produced in this monkey by superinfection in a manner similar to that described in the last experiment.

History of earlier infection. See Experiment (d).

History of superinfection. The animal was superinfected on the 99th day of its infection, with blood from both Monkey No. 7 and Monkey No. 29. These two animals had chronic infections, as the result of infection and superinfection with 7 different strains of *P. knowlesi* and also with *P. cynomolgi* [vide footnote to Experiment (g)].

Parasites detected in scanty numbers on 108th day; these increased in numbers causing a distinct parasitic attack due to *P. knowlesi* with its maximum on 111th and 112th days. No clinical symptoms observed. The latter parasite then diminished in numbers and was replaced by *P. cynomolgi*, which caused a distinct parasitic

*The earlier details of the histories of the infections in these three monkeys are given by Mulligan and Sinton (1933a), and their later histories by Sinton and Harbhagwan (1935) [vide their Experiments (v), (vi) and (ix)].

attack with its maximum from 118th to 121st days. The numbers of this parasite then decreased for a few days but a distinct parasitic relapse again occurred from 128th to 132nd days. The infection then fell to scanty numbers until 149th day, when it diminished for a few days to increase again to scanty numbers. *P. cynomolgi* remained in this degree of prevalence till 166th day, after which no parasites could be detected for 2 days. A scanty mixed infection, mainly *P. knowlesi*, occurred up to 180th day, after which *P. knowlesi* alone was found till 188th day, to be succeeded by a scanty or very scanty *cynomolgi* infection lasting to 210th day. Weekly examinations made after this day showed on most occasions the same parasite in very or extremely scanty numbers up to 318th day. Later examinations, made weekly or oftener up to 606th day, only showed parasites once (on 340th day) and those in extremely scanty numbers.

Result.—Superinfection of multiple strains of *P. knowlesi* and of *P. cynomolgi* into a monkey having a simple chronic infection with *P. knowlesi* (strain K₄) only, produced a distinct parasite attack of *P. knowlesi* followed by a similar attack due to *P. cynomolgi*. The latter infection relapsed later and was succeeded by a low grade prevalence of *P. cynomolgi* which persisted for about 7 months longer. After this time, no parasites could be detected during a further observation period of about 8 months. At no time could any noticeable symptoms be detected as the result of infection.

(B) DAILY INOCULATIONS WITH DIFFERENT STRAINS OF *P. KNOWLESI*.

In the previous experiments [(g) and (h)] it was found that the inoculation of blood from animals infected with several different strains of *P. knowlesi*, produced a distinct parasitic attack. As it was impossible to be certain that all these strains had survived in the donor animals during the long periods in which the superinfection experiments were in progress, it was decided to inoculate another animal with different strains from known infected specimens of *S. rhesus*.

Experiment (i). Monkey No. 570 (*S. irus*).

History of period of observation. This animal showed no parasites at weekly blood examinations during a period of almost 6 months, nor did a normal specimen of *S. rhesus* inoculated with its blood develop infection. At the end of this time the monkey was given a course of anti-malarial treatment similar to that already described in Experiment (b). Observation by weekly blood examinations was then continued for another 3 months, making a total of 9½ months in all.

History of infection. This monkey was then inoculated by the intraperitoneal route with the doses and strains of *P. knowlesi* shown below—

1st day.	Strain K ₂ (Monkey No. 554)	..	20	million parasites.
2nd day.	Strain K ₂ (Monkey No. 559)	..	11'25	" "
3rd day.	Strain K ₄ (Monkey No. 540)	..	0'4	" "
4th day.	Strain C (Monkey No. 244)	..	3'12	" "
5th day.	Strain K ₁ (Monkey No. 55)	..	0'3	" "
6th day.	Strain K ₂ (Monkey No. 128)	..	0'4	" "

TOTAL IN 6 DAYS .. 355 million parasites.

Parasites first detected in very scanty numbers on 6th day after primary inoculation, and showed no increase up to 11th day. From 12th to 46th days they were detected in scanty numbers, except during 4 days when they were very scanty. From this time onward they were found daily in very scanty numbers up to 100th day, except for an increase to scanty numbers on 4 occasions. Weekly blood examinations made for a further 5 months (up to 254th day) showed very scanty parasites on each occasion.

Result.—A mild but very prolonged parasitic infection with *P. knowlesi* developed. No clinical disturbance was noticed.

(C) DISCUSSION OF THE EFFECTS OF INOCULATION OF MULTIPLE STRAINS OF *P. KNOWLESI* INTO APPARENTLY NON-INFECTED SPECIMENS OF *S. IRUS*.

The results of Experiments (g), (h) and (i) show that the injection of many heterologous strains of *P. knowlesi*, either in single small doses or multiple daily ones, failed to produce any marked pathogenic manifestations in specimens of *S. irus* that were apparently free from infection prior to the inoculations.

These findings support the belief that the tolerance observed in such animals is probably due mainly to some innate characteristic and not to an acquired one.

DISCUSSION OF RESULTS OF EXPERIMENTS.

The results of the experiments detailed above show that the type of young, sexually immature specimen of *S. irus* available in our laboratories does not possess any absolute immunity to infection with *P. knowlesi*. On the other hand, as compared with *S. rhesus*, they possess a high degree of tolerance to the pathogenic effects of such infection.

That these differences between the two species of the same genus of monkey, are not due to any lowered virulence of the parasites injected, is shown by the fact that blood inoculated from the same infected animal at the same time will produce a severe and almost invariably fatal infection in *S. rhesus*, while in *S. irus* the infection is accompanied by no noticeable pathogenic effects.

The differences noted would, therefore, appear to be due either to (a) some inherent qualities in the two species of monkey, or (b) to the presence of some acquired tolerance in *S. irus*, or (c) a combination of these factors.

It has not been possible to obtain specimens of *S. irus* bred and living under conditions in which the possibility of the acquisition of some malarial tolerance, as the result of prior infection, could be excluded. We have, therefore, been unable to determine with certainty that the observed tolerance was due to some inherent characteristic of the species. It has, therefore, been necessary to investigate in how far the tolerance could be attributed to an acquired resistance.

That the tolerance might be mainly an acquired one, was suggested by the fact that the animals used in our work were apparently obtained from localities where simian malarial infections are very common (Sinton and Mulligan, 1933c).

'As many of the species of monkey used in experimental work have come from localities where natural malarial infections are not uncommon, it is very difficult to assess how much of any apparent tolerance is natural, and how much has been acquired as the result of previous infection in nature. As was pointed out by Blanchard and Langeron (1912), one is completely ignorant of what are the pathogenic effects produced by these malarial infections in nature, and of the number of deaths they may cause in such conditions'.

'Kossell (1899) reports that parasites are rarely seen in older monkeys. Gonder and Berenberg-Gossler (1908) found few clinical symptoms in natural infections studied by them, but transmission of the infection to young animals produced a heavy parasitic infection. Macfie (1928) reports severe clinical symptoms in a natural infection in a young baboon. Clark (1930, 1931) has pointed out that, among the monkeys of Panama, the infections are most prevalent in infant and juvenile specimens. Green (1932) states that he found natural infections only in young specimens of *S. irus* in Malaya. Our experience with this species of monkey has been similar to that of Green'.

'None of the infected monkeys observed by Seidelin and Connal (1914) showed any clinical symptoms. These workers have suggested that in nature monkeys may show similar conditions to those seen among the local inhabitants in West Africa, i.e., "where practically all individuals are infected with malaria at an early age, thus acquiring for life a more or less marked immunity".'

'It seems very probable that most of the species of monkey, which have been found to have high infection rates in nature, acquire infection in early life. Under such circumstances, the young animals either die or develop a more or less effective tolerance to the pathogenic manifestations of the disease. In some cases this tolerance may be broken down under the stress of adverse circumstances, or when the animal is reinfected with a heterologous strain of parasite. As the animals increase in age, they probably acquire, by continued reinfection, a tolerance to all the local strains and species of parasite. Whether continued weeding-out of the more susceptible animals by deaths from acute infection gradually tends to produce a race of animal which is born with a certain amount of natural immunity is uncertain. Such a state of affairs seems very possible'.*

'One seldom sees very young monkeys in laboratory work, unless these be captured locally. This is probably because the vicissitudes of capture, confinement and transport kill off the weaklings. Such adverse conditions probably reactivate any latent infections in animals with slighter degrees of tolerance, and death may ensue during the relapses in many instances, because of the unnatural conditions of early captive life. It seems probable, therefore, that the majority of animals used in laboratories, in temperate climates at least, are those which have either had an infection, or have developed a considerable degree of tolerance to any malarial infection previously contracted'. . . . 'It is very difficult to determine how much of the tolerance, shown by some species of monkey in the laboratory, is due to natural immunity and how much to tolerance acquired as the result of previous infection (Sinton and Mulligan, 1933c)'.

In such circumstances, it would not be surprising if many of our specimens of *S. irus* had acquired, and still retained, a high degree of tolerance as the result of infection acquired in early life prior to capture.

The tolerance of an animal to infection is dependent upon the balance between the virulence, aggressivity or infectivity of the invading parasite and the degree of resistance, tolerance or immunity possessed by the invaded host to counteract either the multiplication of the parasite, or to neutralise the effects produced by its 'toxins'.

If the observed tolerance be mainly an acquired one, it might be expected that it could be overcome, or markedly diminished, by one of the following procedures—(1) an increased dosage of infection, or (2) an increased virulence of the infecting dose, or (3) a diminution in the resistance or tolerance of the host, or (4) a combination of these.

If, on the other hand, the tolerance were mainly an innate or inherited characteristic of the species of animal, the chances of causing a marked decrease of the tolerance in the intact animal† would be much less likely than with an acquired resistance.

(A) INCREASED DOSAGE OF INFECTION.

In Experiment (e) a total of about 36 million parasites (K_2 strain) was given by six daily injections. In a similar experiment [(i)] about the same

* This hypothesis is discussed in greater detail in a later paper by Sinton and Harbhagwan (1935) (pp. 325–327).

† As noted on p. 503 the tolerance may be lost in splenectomised animals, while Malamos (1934) finds that similar effects can be produced if the reticulo-endothelial system is thrown out of action by blockage.

total number of parasites were given from six different strains of *P. knowlesi*. In Experiment (f) a total of about 43 million parasites (strain C) were given in a single day by three different routes—the subcutaneous, the intraperitoneal and the intravenous.

In none of these experiments was more than a mild parasitic infection produced. No noticeable clinical manifestations were observed.

(B) INCREASED VIRULENCE OF INFECTING DOSE.

It has been found that the virulence of certain pathogenic protozoa may be enhanced by passage through experimental animals. There appears to be no agreement upon this point in regard to the human malarial parasites*. It has been found, however, that very considerable difference may occur in the virulence of certain strains of human *Plasmodia**, as judged by the symptoms produced.

(a) INCREASED VIRULENCE PRODUCED BY FREQUENT PASSAGE.

Napier and Campbell (1932) and Knowles and Das Gupta (1932) believe that their strain of *P. knowlesi* acquired an enhanced virulence, as the result of repeated passage through a very susceptible host, *S. rhesus*. Sinton and Mulligan (1933c) were, however, unable to arrive at a definite conclusion in the matter, because the very virulent effects produced by this parasite in *S. rhesus* did not lend themselves to any very exact evaluation of minor differences in the pathogenic effects produced.

While there is a possibility that the virulence of *P. knowlesi* may be enhanced by repeated passage through *S. rhesus*, we were unable to find that such a suggested increase of virulence had any marked effect in lowering the tolerance of *S. irus*, as judged by the pathogenic effects produced by freshly isolated strains compared with the same ones after being passaged during a period of about 3 years.

(b) INOCULATION OF NATURALLY MORE VIRULENT STRAINS.

Of the seven strains of *P. knowlesi* used in our work on immunity, strain K₁ appears to be the most virulent, as judged by the fact that the relapses following primary infections were found to be more severe and more frequent, and to require therapeutic control more often to save the life of the animal, than in infections with any of the other strains of this parasite. It was decided, therefore, to see whether the tolerance in *S. irus* could be broken down by inoculation with this strain.

As is shown in the results of Experiment (d), while the parasitic attack produced by the inoculation of this strain was slightly more marked than those obtained with other strains, yet there was no noticeable disturbance of the health of the infected animal. In addition, when this strain was inoculated in conjunction with many other strains in Experiments (g) and (i), a somewhat similar result was obtained.

These results show that the observed tolerance in *S. irus* is capable of withstanding infection with the most virulent strain of *P. knowlesi* that was available in our laboratories.

*These points have been discussed at some length by Mulligan and Sinton (1933) (pp. 534-539).

(c) INOCULATION WITH MULTIPLE STRAINS.

Mulligan and Sinton (1933a) and Sinton and Harbhagwan (1935) have suggested that the fatal result obtained in Experiment (vii) of their heterologous superinfections may have been due to the combined effects of a fresh infection superimposed upon a relapse of a previous heterologous one. For this reason, and because it appeared probable that simultaneous infections with a large number of different strains might have a greater pathogenic effect than any one singly, it was decided to inoculate an animal with multiple strains.

In Experiments (g), (h) and (i) seven immunologically different strains of *P. knowlesi* and two of *P. cynomolgi* were inoculated, either simultaneously or within a few days, into 3 different specimens of *S. irus*. Although the parasitic attacks produced were slightly more marked than usual, no appreciable clinical symptoms were detected.

(C) DIMINISHED RESISTANCE OR TOLERANCE OF THE HOST.

If the tolerance observed in *S. irus* was entirely an acquired one, we should not expect, in our present state of knowledge, that—

(a) it would be protective against a very large number of heterologous strains of *P. knowlesi*, or

(b) that it would persist at a high level for a long period after all infection had been eliminated.

(a) INOCULATION OF STRAINS WITH DIFFERENT IMMUNOLOGICAL PROPERTIES.

It has been shown by Mulligan and Sinton (1933, 1933a) that the tolerance acquired in infections with *P. knowlesi* in *S. rhesus* is largely active against superinfections with homologous strains only. In these circumstances, one would not have expected that a tolerance acquired in nature would be protective against all the different strains used in the above experiments. These seven strains, differing from each other immunologically, were isolated from 5 separate batches of monkeys (*S. irus*) purchased at different times during a period of over a year. While it is possible that all the specimens of this species of monkey used in our work may have originated in the same locality, and so been exposed in nature to infections with all these strains, such an event does not seem probable.

The results of the experiments detailed above show that the inoculation, either alone or in combination, of seven different strains of *P. knowlesi* have failed to break the tolerance observed in our specimens of *S. irus*. The experiments of Malamos (1934) in Europe with a strain which was probably heterologous to his animals*, also failed to produce a breaking-down of this tolerance.

These findings support the view that the tolerance seen in *S. irus* is not entirely, or mainly, an acquired one.

(b) INOCULATION IN APPARENTLY NON-INFECTED ANIMALS.

Although there is no positive experimental proof, there is much evidence of various kinds to suggest that the tolerance that is acquired to malarial infections lasts but a comparatively short period after the parasites have been

* Vide footnote, p. 503.

completely eradicated from the host*. On the other hand, such tolerance may persist for very long periods in the presence of a latent or sub-patent infection ('premunity').

In view of the frequency of natural infections in *S. irus* (vide Sinton and Mulligan, 1933c), it is possible that the tolerance observed in specimens of this species depends upon either (i) a 'residual' tolerance remaining after a cured infection or (ii) a 'concomitant' tolerance or 'premunity' dependent upon the presence of an undetected latent infection.

To exclude the presence of either of these conditions in our experiments, the only method which appeared feasible was to keep the animals under observation for very long periods. During these they were examined exhaustively by several different methods for the presence of infection, and most of them were also given an intensive course of anti-plasmodial treatment to eliminate any infection which might have escaped detection by the former methods.

While some of the animals in our investigations were only observed for periods of less than 6 months [Experiments (a), (f), (g) and (h)], the animals used in Experiments (b), (c), (d), (e) and (i) were observed very closely for 22, 22, 13, 10 and 9½ months respectively before inoculation. During this time they showed no evidence of infection and lived under conditions which excluded any chance of the acquisition of fresh infection.

Although these animals were inoculated with various strains and dosages of parasites, the infections developed were all relatively mild, as compared with those seen in *S. rhesus*, and in no case did these have any noticeable effect on the health of the infected animals.

These results suggest either—

(i) that the animals had undetected sub-patent infections which lasted during the period of observation. This appears highly improbable, in view of the methods used to discover such infections and the thorough course of treatment given in most of these cases. Nor would one have expected that the tolerance due to such an undetected sub-patent infection would have remained so effective against all the different strains of parasite used,

(ii) that the tolerance acquired to *P. knowlesi* infections will persist at a very high level for much longer periods than is at present believed*. It seems unlikely, however, that this would remain so effective against all the different strains used, or

(iii) that the observed tolerance is mainly dependent upon some innate characteristic of the race of *S. irus* used in our experiments.

While it is impossible to exclude the presence of 'residual' tolerance as the cause of the mild results observed in the animals inoculated after long periods of observation, it appears more probable, from the evidence available in these and the other experiments, that the condition is mainly due to some

* The duration of acquired immunity is believed by some observers to vary with the species of *Plasmodium*. James (1935) records a patient who continued to maintain a very effective tolerance, or rather an immunity to infection with *P. vivax*, for a period of some years after all parasites of the original infection had apparently been eradicated. It may be, therefore, that the tolerance produced by some species or strains of *Plasmodium* may have a much longer duration than is at present suspected.

innate or racial characteristic of the specimens of *S. irus* obtainable in our laboratories.

(D) COMBINED EFFORT TO LOWER TOLERANCE.

In Experiment (i) there has been a combined attempt both to increase the pathogenicity of the parasite and to lower the tolerance of the host. The animal was inoculated on each of 6 consecutive days with a different strain of parasite. This was an effort not only to increase the dosage of infection but also to raise its potential virulence. The monkey had also been under observation for 9½ months before inoculation, during which time it also received a thorough course of treatment. This should have given an opportunity for the loss, or at least a diminution, of any residual or any concomitant tolerance*.

The fact that these combined methods failed to produce any marked reduction in tolerance is in favour of the view that this is due mainly to some innate characteristic of the monkey, rather than to an acquired one.

(E) GENERAL AND PRACTICAL CONSIDERATION OF RESULTS.

Although many different methods have been used in attempts to destroy, or markedly diminish, the degree of tolerance to infection with *P. knowlesi* observed in the specimens of *S. irus* available in laboratories in India, these have so far been unsuccessful.

The results obtained mainly support the view that this tolerance either represents an existing natural or innate defensive or protective power in *S. irus*, or the ability to develop such a power so rapidly that the effects of infection are controlled before they can reach any such high degree of pathogenicity as that which occurs in the Indian type of *S. rhesus*. It has, however, been impossible to exclude with absolute certainty the possibility that infection with *P. knowlesi* may leave behind it, after radical cure, a residual acquired tolerance, which may persist at a high level for a much longer time, possibly for years, than the evidence at present available suggests as probable.

It is known, as the result of the work on therapeutic malaria, that *individuals* who have never previously suffered from malaria, may vary very markedly in their clinical reactions to inoculation of either *P. vivax* or *P. falciparum*. There is also much evidence to suggest that certain human races are much more tolerant than others to the pathogenic action of malarial infections.

Sinton and Harbhagwan (1935) (pp. 326-327) have discussed the suggestion that a race of individuals, with a high degree of natural tolerance, might be evolved in very malarious areas by a process of 'survival of the fittest', i.e., the more resistant type of individual survives and transmits his characteristic resistance to his offspring. It is not improbable that a similar process of evolution may have occurred in the case of *S. irus*, which species has inhabited for countless ages an area where simian malaria is apparently very highly endemic. One would not expect such tolerance among specimens of *S. rhesus*

*In an addendum to this paper the results of a heterologous superinfection produced a very long time after splenectomy, are recorded. From these it is seen that, if the reticulo-endothelial system be given sufficient time to recover, the inoculation of such a very virulent heterologous strain of *P. knowlesi* as K₄ failed to break down the tolerance of the experimental animal.

from Northern India, where the absence of any noticeable degree of simian malaria would prevent such an elimination of the more susceptible individuals*. As in certain human races, we have on several occasions noted individuals of the latter species of monkey, which appeared to have a greater resistance, or a lesser susceptibility, than normal, to the pathogenic effects of infection with *P. knowlesi*.

The evidence accumulated during the last half century suggests very strongly that certain races of man, inhabiting malarious tracts in the tropics, possess a higher degree of natural or innate tolerance to the pathogenic effects of malarial infection, or the power to develop a tolerance more rapidly and to a higher degree of efficacy, than exists in many other races. If this be so, the observation would be of great practical importance in relation to the employment of 'salted' labour for work in malarious regions of the tropics.

The term 'salted labour', as at present used, appears to include two very different types of population:—

(i) individuals possessing a high degree of natural or innate tolerance, which is usually largely reinforced by an acquired immunity to certain strains and species of parasite; and

(ii) individuals possessing as a race little or no natural tolerance, but merely 'salted' by an acquired resistance to the local strains and species of parasite in their immediate environment.

Labour forces composed of individuals of the former type, when introduced into a strange malarial environment, may suffer to some extent from malarial sickness, due to fresh infections with heterologous species or strains of parasite and to abnormal conditions conducive to relapses of older infections. The intensity and prevalence of such sickness is probably never so severe as in the latter type of population, who have no high degree of natural resistance to reinforce their acquired one.

If the force be not merely a temporary 'tropical aggregation of labour', it is even more important that it should be composed of a high proportion of naturally tolerant individuals, if possible. This is especially the case when such labour cannot be protected adequately against the risk of malarial infection.

The labour problem, which malarial sickness gives rise to in industrial undertakings in the malarious parts of India and other tropical countries, has been discussed in some detail by Sinton (1935a). If one takes the tea industry as an example, these concerns experience great difficulty and expense in obtaining and retaining an efficient labour staff by recruitment. For many years it has been the ambition of the promoters of many such enterprises to establish a domiciled labour force on the spot, from the natural increase of which sufficient labour would be available to meet the needs of future years. Such attempts have usually proved unsuccessful and been hampered by the low number of living births and the high infantile mortality, which are mainly due to the direct and indirect results of malarial prevalence (Sinton, 1935, 1935a).

* 'Infection without symptoms is supposed to be the result of long periods of association (between the parasite and the host). According to this view the length of parasitism of a certain species of host by a certain species of parasite can be determined approximately by the host-parasite reactions. For example, if a parasite is pathogenic and lethal for a certain host the association is supposed to be recently acquired, whereas the absence of symptoms indicates a long period of consociation' (Hegner, 1926).

While much of the labour employed is 'salted', this 'salting' is often merely in the nature of an acquired tolerance with little or no natural element of protection. Under these conditions, one would not expect this acquired character to be transmitted to offspring*, as would possibly be, and probably is, the case in a natural tolerance. It would appear advisable, therefore, when choosing labour for such highly malarious areas where little or no effective protection exists against infection, that the choice of individuals to be recruited should fall rather upon the members of those races or communities in which a natural tolerance is known to be present than upon those who are merely 'salted' by an acquired immunity.

SUMMARY AND CONCLUSIONS.

While the specimens of *S. irus* available in laboratories in India will develop a parasitic infection after inoculation with *P. knowlesi*, they exhibit a high degree of tolerance to the recognised pathogenic effects of infection with this parasite.

Attempts have been made to discover whether the observed tolerance is a natural characteristic of this species of animal, whether, on the other hand, it is a result of an acquired immunity, or whether it is a combination of these two factors.

All efforts to destroy or markedly diminish this tolerance by (i) an increased dosage of infection, (ii) an increased virulence of the infecting dose, (iii) a diminution in any acquired resistance or tolerance in the host, and (iv) a combination of these methods, have proved unsuccessful.

The results obtained have been discussed in detail, and, from the evidence at present available, it appears probable that the observed tolerance of the type of *S. irus* used in the work is a natural characteristic of these animals. It has not been possible, however, to exclude with certainty the possibility that some degree of protracted 'residual' tolerance, resulting from a prior radically-cured infection, may have been partly responsible. This appears to be unlikely as the chief cause of the tolerance.

The question of 'salted' labour in relation to the occurrence of natural or innate tolerance has been discussed, and it has been pointed out that the best type of such labour is probably one in which the individuals possess a natural tolerance and not merely an acquired one.

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* The susceptibility of *S. rhesus* to *P. knowlesi* is apparently transmitted from parent to child. Similarly Van Nitsen (1933) reports that the natives of Ruanda who emigrate to Katanga show a greater susceptibility to malaria than do other tribes of the Congo. He finds that this trait appears to be transmitted to their children born in Katanga.

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ADDENDUM.

The experiments of Sinton and Mulligan (1933b), of Krishnan, Smith and Lal (1933), and of Malamos (1934) show that

(i) if specimens of *S. irus*, infected with *P. knowlesi*, be splenectomised, they will develop acute malarial attacks very similar to those seen in normal specimens of *S. rhesus*, when the latter species is inoculated with this parasite*;

(ii) infections with *P. cynomolgi* are also much more severe in splenectomised than in normal animals of both species; and

(iii) if normal specimens of *S. irus* be splenectomised and kept for several weeks afterwards, to allow them to overcome the shock of the operation, they will still develop acute attacks when inoculated with *P. knowlesi*.

These findings suggest that, if any circulating 'antibody' be present in the peripheral blood, either (i) it quickly disappears or loses its efficacy after the spleen is removed, or (ii) it can only act in combination with large amounts of the type of tissue which is present in the spleen. The latter action might be due either to the removal of some adjuvant substance which is produced mainly by certain parts of the splenic tissue in the normal animal, or because the phagocytic action of the splenic tissues in the intact animal is needed to make the protective 'antibody' fully effective against such a severe infection as that caused by *P. knowlesi*, or to both factors.

When the spleen is removed the animal is depleted of a very large amount of its reticulo-endothelial tissue. It is known, however, that after splenectomy

* Malamos (1934) also records a similar action after 'blockage' of the reticulo-endothelial system in *S. irus*.

there gradually occurs a compensatory hypertrophy of this tissue in other centres to balance the loss. If the tolerance, either natural or acquired, shown by certain monkeys against *P. knowlesi*, were dependent mainly upon the reticulo-endothelial system, one would expect that, when the compensatory hypertrophy had fully developed, the tolerance to infection would also tend to return towards the level shown before splenectomy. An experiment was carried out to investigate this point, and at the same time to test the duration of the 'natural' tolerance of *S. irus*.

Experiment (j). Monkey No. 56 (*S. irus*).

This animal was the natural host of strain K₃ of *P. knowlesi*. Continued blood examinations showed that it was also infected with both *P. cynomolgi* and *P. inui*.

History of infection. Parasites present at daily blood examinations for 6 months after purchase, and afterwards at weekly ones for nearly six months more. After a total observation period of about a year, *splenectomy* was performed. This was followed immediately by a very acute attack due to *P. knowlesi*, requiring treatment to save the life of the animal. Several very acute relapses occurred during the next 9 months, and these also required treatment. The later attacks were, however, less severe than the earlier ones. Afterwards a latent infection lasted for 14 months, during which weekly blood examinations either showed no parasites or only very scanty numbers, sometimes *P. knowlesi* and sometimes a mixed infection. During the succeeding 4 months, no parasites were found at such examinations. The animal had now been under observation for 39 months in all, and 27 months after splenectomy.

It was then *superinfected* with a heterologous strain of *P. knowlesi* (K₄) which produced only a very mild parasitic infection.

Result.—Splenectomy caused a very severe and immediate attack due to *P. knowlesi* (strain K₃), in a specimen of *S. irus* which had previously shown a high degree of tolerance to this strain of parasite. This was followed by a series of severe relapses, requiring treatment but diminishing in severity during a period of 9 months. For the next 14 months a latent infection could be detected, but no parasites could be found after this up to the end of 27 months after splenectomy. Superinfection with a heterologous strain of *P. knowlesi* (K₄) produced only a very mild parasitic attack.

DISCUSSION.

The immediate loss of both its 'natural' and its acquired tolerance by this splenectomised animal against K₃ strain of *P. knowlesi*, suggests that in an intact animal the tissues of the spleen have a close relationship to this aspect of immunology in simian malaria. The tissue concerned is almost certainly the reticulo-endothelial system, the bulk of which is contained in the spleen of the normal animal. That the residue of this system, left after splenectomy, is unable to cope with the infection is shown by the acute attack which occurs immediately, and also by the succeeding relapses which occur for a considerable period after the operation. The fact that these relapses tend to diminish in their intensity, suggests that, as the compensatory hypertrophy of the reticulo-endothelial elements of other tissues increases in amount, so does the power of the animal to develop a higher degree of tolerance. When ample time had been given for this hypertrophy to replace to a large extent the tissue lost by

removal of the spleen, it was found that both the 'natural' and the acquired tolerance of this animal had again risen to a high level.

This result suggests very strongly that both the 'natural' and the acquired tolerance exhibited by *S. irus* to infection with *P. knowlesi*, are intimately bound up with the splenic functions of normal animals, and probably with the reticulo-endothelial tissue of that organ especially. The results obtained with other animals, either already infected or inoculated within a relatively short period after splenectomy, show that the blood and tissues of the body do not *per se* have such a high degree of protective action as to be capable of preventing an acute infection in the absence of the spleen. This negatives the view that 'natural' tolerance depends upon the blood being an unfavourable medium for the multiplication of the malarial parasite.

The specimen of *S. irus* used in this experiment was the natural host of strain K₃ of *P. knowlesi*. The fact that this strain has been shown to be immunologically heterologous to strain K₁, when inoculated into *S. rhesus*, proves that the original host could not have had an infection with the latter strain when purchased by us. This being so, the animal in Experiment (j) exhibited a high degree of tolerance to strain K₁ after an interval of 39 months, during which it had no chance of maintaining a 'concomitant tolerance' or 'premunity'. This finding supports the view that the tolerance observed in *S. irus* to infections with *P. knowlesi*, is a natural or innate phenomenon and not an acquired characteristic as the result of previous infection. This tolerance would also appear to depend upon the integrity of the reticulo-endothelial system.

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